REPRINTED FROM

THE QUARTERLY JOURNAL OF
MICROSCOPICAL SCIENCE.

The Habits and Early Development of Cerebratulus Lacteus (Verrill).

A Contribution to Physiological Morphology.

By
Chas. B. Wilson.

With Plates 9—11.

OUTLINE.

INTRODUCTORY.

THE ontogeny of the Nemerteans, and more particularly that of the Schizo-nemerteans, is of special interest on account of the metamorphoses through which the young embryos pass.

Ever since its discovery and naming by Müller in 1847 (**38**) the pilidium form of larva has attracted the attention of leading scientists the world over. As a result of their investigations we are to-day in possession of the leading facts with reference to the histology of the pilidium and the metamorphoses from this free-swimming form into that of the adult worm.

Much work has also been done upon the origin and maturation of the sexual products in different species. But our knowledge of fertilisation, segmentation, and the early history of the pilidium has been derived almost wholly from two papers by Metschnikoff, one in 1870 (**35**) and another in 1882 (**36**).[1]

The former paper I have been unable to examine, but from the excellent abstract given by Bürger (**13**) I judge that Metschnikoff obtained the eggs already fertilised, and followed their subsequent development as closely as possible.

At all events he confines his attention in both papers almost exclusively to the later development of the larva, and seems to have derived his facts from observation of histological material, and not from a study of the living embryo.

[1] Since the above was written it has been my pleasure to receive from the author, Dr. W. R. Coe, of the Sheffield Biological Laboratory of Yale University, a copy of his excellent paper upon "The Maturation and Fertilisation of the Egg of Cerebratulus" (**16**). This is the first step toward supplementing our knowledge of this long neglected group and is most praiseworthy.

In the present investigations for the first time, so far as can be determined, the eggs of a Nemertean were fertilised artificially.

The consequent abundance of embryos has rendered it possible to follow in detail every step in their development, and to use living material far more than has ever been done heretofore.

In much of the recent embryological work there has been a tendency to confine the attention almost exclusively to the study of sections and prepared material. And embryos have been reared for the sole purpose of furnishing such material. But it is clearly evident that the true purpose of histology is to supplement, and not to supplant, the study of the living animal. For this reason the morphological drawings in the present paper have been made, whenever it was possible, from living specimens, with the aid of the camera lucida.

Adults have also been captured and kept in aquaria where their habits could be closely watched, and many interesting facts have been thus discovered. Eggs have been laid and fertilised by individuals thus kept in confinement, so that it has been possible to verify all the details obtained from the artificially fertilised eggs, and, in addition, to discover the method of egg-laying and fertilisation.

The development and habits of the pilidium have been carefully watched for four successive summers, and as a result it is possible to present quite a full account of this particular species. A second result has been the growing conclusion that this species is particularly fitted for embryological work in our laboratories. Many different reasons have led to this conclusion, among which may be mentioned:

1. The vitality of the adults, by reason of which uninjured sexual products can be obtained from them even under very adverse circumstances. When reduced to such a dire necessity, a single piece of a disruptured worm, but a few inches in length, can be used at the rate of half an inch a day for a week or more, and it will yield all the while germinal pro-

ducts capable of producing normal embryos. A clean glass vessel (4 to 6 gals.) with plenty of the sand or mud from which the animals were taken, and a constant but slow renewal of the water, has proved to be the best aquarium. Only one pair of worms should be placed in a single vessel, and the supply of plant life should be very meagre. Under these conditions no difficulty has been experienced in keeping the worms for six or eight weeks, and they have laid profusely (cf. Coe, **15**).

2. The ease and uniformity with which the egg may be fertilised artificially.

3. The persistence and remarkable activity of the polar bodies. They remain in close connection with the egg all through segmentation and gastrulation, the second body being actually connected with the blastomeres by protoplasmic processes, while both display to a marked degree those protoplasmic activities which have been designated as "spinning."

4. The transparency of the larva subsequent to gastrulation, which renders it very easy to follow its internal development. It is so clear that very high powers may be successfully used. An excellent opportunity is thus afforded for the study of muscular development, owing to the peculiar nature of the mesenchyme cells.

Every fact of importance has been included in the present paper; when not verified by personal observation, due acknowledgment has been made. The author desires to express his indebtedness to Librarian Wm. I. Fletcher, of Amherst College, for the free use of the science library of that institution, without the help of which the present work would have been impossible. It is likewise a pleasure to acknowledge the inspiration and the many valuable suggestions given by Dr. E. A. Andrews, of Johns Hopkins University, and by Mrs. G. F. Andrews. And to Dr. W. R. Coe, of the Sheffield Biological Laboratory, acknowledgment is due for the kindly loan of mounted material illustrative of spermatogenesis, and for much friendly criticism.

The Adult Worm.

Determination of Species.

The species under consideration is one of the largest of the Nemerteans, and possesses all the characteristics of the group. These consist negatively in the absence of all external appendages and of a definite body-cavity, and the entire lack of visible segmentation. Positively they include the possession of a smooth body, flattened dorso-ventrally, and of a very long tubular proboscis, which can be protruded by eversion from an aperture in the front of the head.

Much work has been done upon the classification of the Nemerteans with no decisive results. Different species and even genera frequently resemble one another so closely that they cannot be distinguished after preservation. Hence we can find in the attitude of different authors every variation from one (**46**) 75 per cent. of whose enumerated species are new creations, to another (**27**) who would include all the Schizo-nemerteans under a single genus. And our present species is classified by different authors under distinct names and even under different genera. The name we have chosen is the one selected by Verrill, but the same species was called by Girard Meckelia fragilis, by Leidy Meckelia ingens, though at first he designated it M. lactea. The name of the group, Nemerteans, was first applied by Cuvier, and is derived from Nemertes, one of the Mediterranean sea-nymphs, daughters of Nereus and Doris.

The generic name, Cerebratulus, was first given by Renier in 1804 to a single species, and continued until recently to be simply a specific name. It is derived from cerebrum, the brain, probably from a fancied resemblance of the tissues (**41**).

The specific name, lacteus, was given by Leidy to certain small white specimens, which afterwards proved to be the young of this species, and accordingly the name was

extended by Verrill, in 1892, to include the larger adults which are often not at all "milky" (**46**).

Habitat and Habits.

This large Nemertean is quite common along the entire Atlantic coast, from Florida to Massachusetts Bay, and is found locally at Casco Bay on the coast of Maine. From this latter locality were obtained all the specimens which furnished data for the present paper. Verrill states (**46**) that he found adults at Quohog Bay, one of the numerous inlets of Casco Bay. These were associated with a number of other southern forms, such as Venus mercenaria, Crepidula convexa, Eupagurus longicarpus, Gammarus mucronatus, Nereis libata, Meckelia ingens, Asterias arenicola, etc. These species properly belong to the region south of Cape Cod, and it might be inferred that Cerebratulus also was a southern species, and that it could be found in northern waters only under specially favourable conditions.

Such, however, is not the case; I obtained a number of specimens from Quohog Bay, but found them more abundant at Stover's Point on the east side of Harpswell Neck. All the specimens used were obtained here, but none of the southern species named above were found anywhere in the vicinity. Furthermore, the Point is only about a mile from the end of the Neck, and it gets the full sweep of the tide from the open ocean twice a day.

The largest colony of Nemerteans was on the outer or exposed side of the Point, and was associated exclusively with hardy northern species. The fishermen often find these worms when digging for clams during the winter, and some of the material used for the origin of the sexual products was obtained from them at that season. When we reflect on the severity of the winter upon the Maine coast, where the mud is exposed between tides to an atmosphere that frequently

registers many degrees below zero, we may conclude that Cerebratulus has at least become well acclimated.

This species burrows in sand and mud between tides, and in shallow water down to several fathoms in depth.

It occurs most plentifully at and just above low-water mark, but may be found under stones in sheltered positions (Quohog Bay), nearly up to the high-water mark of medium tides. It is gregarious in habits, and the finding of one is a good indication that there are others in the immediate vicinity. It also occurs in the same localities year after year, but after prolonged search in any one place the number of large specimens becomes very sensibly diminished. The largest specimens are not solitary, as McIntosh declares to be the case with the great Lineus marinus (**34**), but they are so limited in numbers as to warrant the conclusion that here, as in some of the higher forms of life, size is "inimical to profusion." One of its favourite haunts is along the edges of an old mussel-bed amidst the broken shells and stones, into which it is often almost impossible to drive a clam-hoe. But it is also dug up with clams in clean white sand and mud, and is known locally to the fishermen as a "clam-worm."

Scarcely a trace of the worms save the entrance to their burrows can be seen except at high tide. This is their period of activity, and they may then occasionally be observed gliding amongst the sea-weed on a muddy or sandy shore, or in the shade of the rocks in a large tide-pool. They are essentially nocturnal, and come out of their burrows much more frequently during the night tides than during those which occur in the day time. The burrow itself is little more than a hole or tubular opening through which the worm can move freely in either direction. The walls of this tube are coated thickly with the slimy mucus exuded from the worm's skin, which materially facilitates its progress.

The burrows are neither very long nor very deep, for even the largest worms are found within a few (six or eight) inches of the surface. There is no evidence to show that the same burrow is inhabited for any length of time. On the contrary,

all observations indicate that the worm roams about constantly in the mud after food, and that it is able to move quite rapidly, even when forming a new burrow. The enormous amount of slime secreted by the worm's ectoderm may be inferred from the fact that it keeps the walls of its burrow constantly coated.

This slime often oxidises the iron elements in the mud, with the result that the latter is tinged a dull rusty red in the immediate vicinity of the burrow.

Repeated attempts were made to ascertain just how the burrowing was done, by digging out specimens and throwing them on a fresh surface. It was noticed that the worm thrust its pointed head a little way into the mud and then seemed to brace itself for a further effort, the head at the same time being withdrawn slightly. The significance of this fact was in doubt for some time, but on placing a few worms in a glass aquarium with five or six inches of mud at the bottom, they immediately began to burrow near the glass. It was then seen that the bracing action was due to the fact that the worm was driving its proboscis into the mud ahead of itself. The proboscis was protruded for six or eight inches, and then enlarged slightly and crooked a little at the end. Aided by the hold thus obtained the worm was able to thrust its head rapidly into the mud, keeping it narrow and pointed the while.

The extreme tip of the head was then contracted into a broad rounded form, and the wave of contraction thus started passed slowly backward along the anterior portion of the body, thereby moving the latter forward about an inch. As soon as this wave was fairly started in its backward motion the head became pointed, the proboscis was thrust forward again, and the whole process was repeated. The tip of the head was then contracted, and a second wave was started backward before the first had reached the centre of the body. In this way one wave followed another so quickly that the resultant motion was nearly a steady advance instead of being jerky. This rapid burrowing by means of both proboscis and

body contractions must be of great service to the worm in escaping from its enemies and in obtaining its food. In locomotion the proboscis seems to be of use chiefly as an organ of feeling or touch, being thrust forward to determine the direction in which the burrow is to be made. Whenever it strikes an obstacle, such as a small stone, it feels around it, sometimes on several sides, and selects apparently the place that offers least resistance.

In doing this it often happens that the proboscis will be thrust up into the water. It is then quickly withdrawn only to be thrust up again, perhaps in a very few seconds.

The only idea of direction, therefore, seems to be to keep beneath the surface of the mud. It is also evident that when the proboscis is thrust forward forcibly into the mud or sand, it makes an opening for the subsequent passage of the head.

The worm may often be seen to withdraw its proboscis and push its head along in the opening thus left. It is also possible that the proboscis may have some tractile power in helping to pull the body forward. That it is actually used as an organ of prehension was witnessed several times.

The Nemerteans were fed with common clam-worms (Nereis). When a Nereis in wriggling about came in contact with a Nemertean, or crossed the mouth of a burrow when a Nemertean was inside, the latter suddenly darted out its proboscis and coiled it spirally around the Nereis. The spiral coil covered an inch or more of the Nereis's body, and the proboscis was then slowly withdrawn, bringing the Nereis up to the Nemertean's mouth, where it was finally swallowed. This corresponds exactly with the graphic description given by Kingsley (cf. **34**) of a specimen of the species Polia mandilla (Amphiporus lactifloreus, McIntosh) in the act of devouring a fish. McIntosh, however, questioned Kingsley's accuracy (**34**), because the latter says that the proboscis assists in prehension, but Cerebratulus certainly uses it sometimes for that purpose.

When withdrawn the proboscis is coiled up in that portion

of the sheath which lies above the œsophagus, as can be seen in fig. 4.

Its large anterior end is continuous with the walls of the sheath just in front of the ganglionic commissure, while its small posterior end is fastened at a single point where the œsophagus joins the intestine. As the proboscis itself is several times the length between these two points of attachment it is coiled very closely. Furthermore, being attached at the posterior end, it follows that it must always be of double thickness, with a blunt anterior end when everted. This increases its strength for prehension and burrowing, and does not seem to detract from its delicacy as an organ of touch.

Food.—This species of Cerebratulus is almost wholly carnivorous, and feeds upon other worms which frequent the mud between tides, showing a decided preference for Nereis.

Several times both Nereis and Nemerteans were placed in the same dish when obtained; the former were found to have been swallowed by the latter on reaching the laboratory half an hour later. The Nereis is always swallowed tail first (fig. 1), which is exactly the opposite of the method usually followed by carnivorous animals, but is occasionally found in other carnivora, as when a snake swallows a frog or toad (**23**).

In such cases the animal swallowed is usually defenceless, while its captor is well armed, but in the present instance these conditions are exactly reversed. And it is very hard to understand how a Nemertean, which has neither teeth nor jaws, nor indeed offensive weapons of any kind, can yet overcome a well-armed Annelid nearly as large as itself. The head of the Nereis, with its powerful jaws, is left entirely free, but although I have seen the victim wriggle frantically in its efforts to escape, until it finally disappeared down its captor's throat, it never made any attempt to bite. This is the more remarkable in view of the well-known fact that when two Nereis come together they almost invariably fall upon each other tooth and claw, and often inflict very severe

wounds with their powerful jaws. Dr. E. A. Andrews has suggested to me that possibly the dense coating of slime which is constantly exuded from the Nemertean's ectoderm may afford it protection. If a minute drop of this slime be placed upon the tongue it will be found so intensely acrid as to parch the whole mouth, and the taste remains for a long time. This quality, then, might effectually prevent the Nereis from inflicting any injury with its jaws. These Annelids do not often leave their burrows altogether, but simply protrude the head and anterior portion of the body in search of prey. Taken in connection with the fact that the Nemertean swallows them tail first, this suggests that they are not ordinarily caught outside their burrows, but that the Nemertean probes around beneath the surface until it strikes the home of an Annelid, and then proceeds to swallow the unfortunate occupant. In such a case the Annelid's jaws would be practically useless, for it could not turn about in its burrow. The snout of the Nemertean, in front of the mouth, is usually turned backward during the process of swallowing, the proboscis being wholly withdrawn.

The digestive fluid acts very quickly. Several attempts were made to preserve a specimen with a Nereis nearly swallowed and protruding an inch or two from its mouth. But they failed at first because the Nereis was forcibly ejected as soon as the Nemertean touched the preserving fluid, no matter how far it had been swallowed. A subsequent attempt was crowned with success; a Nemertean with an inch of Nereis nearly as large as itself protruding from its mouth was plunged into boiling water for a moment and then preserved in formalin. This produced death so instantaneously that there was no time for ejection, and the perfectly preserved pair still remain to attest the fact (fig. 1).

But although the first efforts were failures in one direction they were a success in another. The swallowing of an ordinary Nereis occupies about ten minutes, but in every instance it was found more than half digested upon ejection.

The same thing happened when a dead squid was left in a pail with some Nemerteans. One of them swallowed a long arm of the squid, and when its further progress was stopped at the base of the arm it drew its body down in wrinkles until as much of the digestive tube as possible had come in contact with the arm. It remained in this position for five or six minutes and then withdrew, leaving the arm completely digested for more than half its length. Of course, there is very little substance to the body of a Nereis, and it can be assimilated without much change; but the arm of a squid is more substantial, and must require considerable digestion. It is evident that this takes place rapidly at the very beginning of the alimentary canal. This fact suggests one reason why the loss of the posterior portion of the body does not seriously affect the animal.

Several of the fishermen have told me that specimens of Cerebratulus are sometimes caught by them when fishing in shallow water with a bait of Buccinum undatum or Natica heros.

The same thing is noted by McIntosh (**34**) with reference to the deep-sea form, Lineus marinus, which was caught while fishing for cod. Like that species, Cerebratulus also must have "an indiscriminate appetite," feeding upon both living and dead forms, and ejecting the bristles and other indigestible material through the anus. Occasionally a bristled or spiny victim proves too much for its captor, with the result that the bristles or spines perforate the digestive tract and the body-wall.

Dr. W. R. Coe tells me that he found a Cerebratulus in this condition with a large Nereis half swallowed, the posterior portion of the Nereis protruding through a large rent in the ventral wall of the œsophagus. In confinement Cerebratulus eats readily almost any animal food, but prefers a Nereis to anything else.

Breathing.—This species and probably most other Nemerteans breathe by means of the walls of the œsophagus.

With the incoming tide the Nemertean opens the anterior

end of its burrow by turning it abruptly upward to the surface, and then lies with its head in the lower part of this vertical portion. The burrow around the head and in the immediate vicinity is enlarged somewhat, so that the water can circulate freely, and by alternately swallowing and ejecting mouthfuls of water the Nemertean generates a very perceptible current.

The swallowing is long and slow, while the ejection is short and abrupt, the two together occupying about ten seconds. This is evidently the Nemertean's mode of breathing, and the purification of the blood must take place in the walls of the œsophagus, as we should naturally infer from the arrangement of the circulatory system. These facts, of course, were chiefly gathered from specimens kept in an aquarium where the supply of water was constant, but it was curious to note that they alternated periods of rest with periods of breathing exactly as if they had been subject to the flow of the tide. And their periods of rest usually, though not always, corresponded closely with those of the ebb tide.

During the breathing the cephalic slits along the sides of the head were occasionally, though not regularly, opened and closed. At such times, of course, a current of water would enter the side-organs, and it is possible that they assist somewhat in the purification of the blood. But from the regularity of movement in the œsophagus and the entire lack of it in the cephalic slits, we must conclude that the former constitutes the true respiration.

Locomotion.—The mode of locomotion by means of which the Nemertean moves through its burrow has already been described.

They also come out of their burrows at times and swim about in the water. Verrill states (**46**) that "while swimming the body is turned up edgewise and thrown into many undulations, and the motion resembles that of an eel, but is less rapid." Coe adds (**15**) that "it is often met with at night swimming near the surface of the water." This is a

well-known habit of all species, the lateral margins of whose bodies are produced into a thin edge throughout the greater part of their length. But although I have been out at night repeatedly and towed over localities where the worms were abundant, I have never been fortunate enough to catch one swimming.

Frequently, however, some that were dug out of the sand were thrown into the water and watched swimming about, and others have often been seen swimming in the aquarium. Under such circumstances the Nemertean does not turn up edgewise, but undulates about in every possible position, the body being now dorsal side up, now ventral, now with the right side uppermost, now the left, the change from one position to another being continuous though slow. It would seem probable that they come out of their burrows and swim about most frequently during the breeding season, although eggs are laid inside the burrows, as we shall see later.

In addition to swimming and crawling through their burrows these Nemerteans are also capable of moving along on the bottom over the mud or stones quite rapidly, and yet as they have no setæ like the earthworm, nor any other means to prevent slipping, and as the surface of their body is slimy, there is always a considerable loss of motion. On a solid surface the mode of progression is by crawling in a manner similar to that of a Gastropod, with the exception that the waves of muscular contraction, passing backward from the head, are much more apparent.

But this is true only of the adults; young individuals can glide over the glass sides of an aquarium so smoothly that their bodies show scarcely a wrinkle (cf. **34**).

The Nemertean also leaves a track of mucus, like the Gastropod. This mucus is exuded even when the animals are lying coiled up at rest, and it is so abundant that a pail containing several fresh vigorous worms is soon filled with a perfect meshwork of mucus, from which the worms must be pulled or cut out. And it acquires sufficient consistency to offer considerable resistance. If it be removed it is quickly

formed again, but several removals produce a marked diminution in the amount secreted. This abundance of mucus gives some of the smaller forms the ability to crawl back downward on the surface of the water, after the manner of nudibranchs, the mucus forming a sort of float, and being firm enough to give the animal a purchase for its muscles. McIntosh states (**34**) that "if a Nemertean is raised from the surface on which it crawls it always clings most pertinaceously by the anterior end; indeed it would appear that the lips exercise a kind of sucker-like action, or at least that the flattened under-surface of the snout does so."

I have never been able to verify this observation on Cerebratulus, for I could not see that one portion clung to the supporting surface any more than another. But the head and anterior œsophagus are usually kept in contact with the surface, while the other portions of the body are often removed temporarily.

Dismemberment.—Almost every scientist who has dealt with the Nemerteans has noted their disagreeable habit of dismembering themselves when irritated. But even the specialists have not gone beyond a mere statement of the fact except in one or two instances, while the best and most recent monograph on the Nemerteans, that published by Bürger as the 22nd volume of the 'Fauna and Flora of the Gulf of Naples,' does not even mention the habit at all.

We are informed that we must secure our specimens very quickly, and kill them in something that produces instantaneous death if we expect to obtain anything more than a handful of fragments for our pains; but we are left to answer for ourselves the many interesting questions that are sure to arise. Do the Nemerteans ever fragment without irritation? If so, under what conditions and what advantage is it to the worm?

After fission can both anterior and posterior fragments regenerate, the one a new body and the other a new head?

In answer to the first question, I had noticed for three

summers that it was very difficult to obtain perfect specimens toward the close of the breeding season, though they were fairly common at an earlier date. On the contrary, the number of specimens possessing regenerating papillæ increased as the season advanced. It was evident, therefore, that they must dismember for some reason during the breeding season, and it was suspected that the eggs might be discharged in this way. During the past summer the matter was put to test with the following results.

A perfect male and female Cerebratulus were secured during the latter part of June, and placed in an aquarium half filled with the same mud from which they were taken. They burrowed into this mud at once, and remained there under fairly normal conditions, and apparently perfectly healthy, until the 1st of September, when they were both preserved on leaving.

They were nearly sexually ripe when obtained, and after being kept a fortnight the female laid three batches of eggs at short intervals, each of which was fertilised by the male.

Soon after the last lot was laid they both dismembered, leaving but a comparatively short piece of the body attached to the œsophagus. This anterior portion at once began to regenerate, and by the 1st of September, an interval of three and a half weeks, it possessed a regenerating papilla nearly 50 mm. long in the female, and about 40 mm. in the male.

The posterior fragments lived for two weeks, and then died with no signs of regeneration. I have kept headless fragments alive, however, under much less favourable circumstances for nearly a month, and McIntosh in his monograph (**34**) states that most of the pieces of a fragmented Lineus were alive six months after dismemberment, and that the sexual products were developed and discharged from them in a perfectly normal manner.

But this happened to be a male, and he could obtain no eggs upon which to test the fertilising power of the sperms.

In another species the posterior fragment began to develop a new head, and he figures several stages in the process, which occupied nearly three months but was not completed.

Barrois also has described the regeneration of headless trunk-pieces in another species, and they have been known to regenerate new heads in the common Lineus socialis.

Fission certainly produces very little apparent effect upon vitality. I have repeatedly obtained headless fragments of both sexes containing unripe sexual products and kept them until they ripened, a period sometimes of two or three weeks, and then fertilised the eggs from one fragment with the sperm from another, and obtained perfectly healthy pilidiums as a result.

Of course the posterior fragments can eat nothing, but the anterior ones display as voracious an appetite as ever immediately after fission, or even during the process. This is attested by the preserved specimen whose photograph is shown in fig. 1. The prolonged retention of sexual functions by headless fragments under adverse circumstances gives some idea of the vitality possessed by these worms in their native haunts, but repeated search has failed to find a single one in which regeneration has even commenced.

These facts, taken in connection with those developed in the aquarium, lead to two conclusions. First, that Cerebratulus frequently dismembers at the close of the breeding season; and second, that while the anterior fragments regularly regenerate, the posterior ones seldom if ever do so.

Professor Benham, of Oxford, has noted a similar case of spontaneous dismemberment in the genus Carinella (**10**).

Here also there was a relation between fission and genital maturity, but of a different nature from that in Cerebratulus.

He found that the genital elements in Carinella were present only in the posterior regions of the body, and that this portion was constricted and cut off as fast as the elements ripened.

Fission in this genus, therefore, would be a method of discharging the ripened sexual products.

In Cerebratulus, on the contrary, the genital elements are distributed throughout the whole body, and if there be any priority in ripening, it is in favour of the anterior and not the posterior portion. Fission does not take place until after the eggs and sperm have been discharged, and hence it cannot be regarded as assisting that discharge in any way.

We are thus confronted again with the question, what advantage can result to the worm in thus parting company with a large portion of its body at this particular time?

The body is composed essentially of two series of pouches, arranged in alternating pairs on either side of the straight intestine. Ordinarily the digestive pouches occupy the larger part of the space, but as the breeding season approaches the reproductive organs begin to enlarge, and by the time the sexual products are ripe they occupy practically the whole of the space, and the intestinal cæca are flattened between them until their opposite sides meet. This is especially true in the posterior portion of the body, which at this time becomes little more than one large ovary or testis, divided into lobes by the flattened cæca. McIntosh (**34**) states that "the glandular elements in the walls of the digestive tract undergo a certain amount of atrophy during the period of reproductive perfection." For a long time, therefore, these intestinal pouches can function very little, if at all, and so they contribute nothing to the nourishment of the body.

After the eggs and sperm have been discharged there is evidently still less use for them until the next breeding season, and regeneration proceeds so fast that before that time a new body will have been formed. In fragmenting, therefore, at the close of the breeding season, it would seem that Cerebratulus is parting company with a portion of its body which has for some time been devoted to a single purpose, that of perfecting the sexual products, and of which, now that this purpose has been accomplished, it has no immediate need.

Fragmentation under irritation is evidently a defensive action. As Hubrecht (**27**) well says, "an animal that at the

approach of danger can separate in two or more parts, each of them capable of regeneration and so of producing an entire new animal, evades this danger very effectively by doing so."

But even if all the fragments are not capable of regeneration, the nemertean still has the chance that its enemy will be satisfied with half a loaf, and that the posterior half.

The actual fission of the body-walls is brought about by internal phenomena which will be discussed later (see p. 118).

The circular muscles at the severed ends contract violently, and draw the margins of the rupture together so tightly that the end is completely closed. It is also rounded like the true anal end of the body, and this resemblance is often increased by a slight emargination at the centre, due to the excessive contraction of the muscles, and by the formation of a regenerating papilla in this emargination (fig. 2).

Such a papilla is slender and almost pure white in colour. At first it is difficult to distinguish it from the true anal papilla with which the body normally terminates, but it may be recognised by the fact that it always possesses a very broad base which fades gradually into the body-wall, while the anal papilla is narrow and ends abruptly at the emargination.

After closing the broken end the circular muscles go into a kind of tetanus, and keep it closed until its edges are joined permanently by the formation of new tissue.

No such formation of new tissue has been observed thus far in the case of the posterior fragments, but their ends remain closed, and this no doubt aids them in retaining their vitality for so long a time. The regeneration papilla rapidly increases in length and width, but always tapers much more than the normal body and remains light-coloured. Specimens have been obtained in which this papilla was four or five inches in length.

Fission takes place through the intestinal cæca, since they offer the point of least resistance, and it usually involves a rupture of the adjacent sexual pouches.

The process is very rapid under irritation, and may be completed within a few seconds after the application of the irritant. It may also take place at two or three points at the same time.

If the irritation be extreme the process may be carried so far that the whole body is divided into very short fragments, the majority of which contain but a single pair of ovaries or testes. This is a frequent result when the worms die through a stagnation of the water in which they are kept.

In such instances the fission goes forward slowly and may occupy several hours, and the contents of the genital pouches are commonly discharged, whatever their state of maturity.

Fresh water acts as a powerful irritant upon Cerebratulus, and always induces dismemberment, but it does not kill very quickly.

Fission and regeneration in Cerebratulus is not confined to the body-walls; it often takes place in the proboscis.

Under the action of powerful irritants both the anterior and posterior connections of the proboscis are severed at once, so that the extruded organ is not turned inside out, but remains in its ordinary condition, or it may be half everted and then extruded bodily. In either case it often lives a long time, many days or even weeks. If it be irritated, and sometimes voluntarily, it moves in such a lifelike manner, and looks so much like a young worm, that it might easily be mistaken for one. Indeed, a similarly extruded proboscis of another species did mislead several excellent zoologists (**6**).

They described it as a young animal, and its possessor as viviparous, and presented both as such to the British Museum, where they now remain as striking examples of the folly of judging by external appearances. A proboscis thus extruded is reproduced by the formation of a small conical papilla on the surface of rupture just in front of the ganglionic commissure. This papilla grows backward rapidly, and a new organ is formed in a few weeks. A similar renewal takes place when the proboscis is forcibly removed by cutting (cf. **33**).

This species, therefore, in common with others, possesses

the ability to restore lost parts so quickly, so easily, and with so little apparent inconvenience during the process, that anything short of being absolutely eaten whole could scarcely prove fatal.

We are left with the two most interesting questions to answer. First, how is fission actually accomplished? The only description of the process that can be found is the one given by Benham (**10**). From a study of sections in different stages of fission he concludes that a double row of nuclei appear in the connective tissue along the line of rupture, and that the longitudinal muscle-fibrils are severed between these rows of nuclei, either through the cell substance of the connective tissue becoming actively contractile and nipping off the fibrils, or in consequence of the fibrils being eaten through by some solvent action.

Neither of these conclusions would explain fission in Cerebratulus, for the whole process, under irritation, occupies only a fraction of a minute. Hence the connective tissue would have no time to become contractile, but must exist in that condition all the while, ready to act at an instant's notice, i. e. it must itself be muscle. On the other hand, any solvent powerful enough to act in so limited a time would not be likely to stop with the mere severing of the longitudinal muscle; it would dissolve everything within reach, and prevent any regeneration. How, then, is fission accomplished?

The rupture takes place through one of the intestinal cæca, where the body-walls can be most easily divided.

It may take place at any cæcum, and sometimes under extreme irritation the worm will divide itself into fragments so small that they contain but a single pair of pouches.

If there were any special apparatus for producing fission it ought to reveal itself opposite each of the cæca, at least in specimens killed after irritation. But careful examination fails to reveal anything of the sort.

What we do find is that Cerebratulus has a thick layer of circular muscles, which are almost entirely lacking in Carinella.

There are also well-developed dorso-ventral muscles in the walls separating the intestinal cæca from the reproductive pouches, and these muscles are enlarged into thick pillars at the inner ends of the walls (fig. 60). Finally, an examination of the regenerating longitudinal muscles shows that in early stages of growth they consist of both longitudinal and transverse fibres, about equally divided (fig. 60). Later the longitudinal fibres become more numerous, but there are always many transverse ones to be found among them.

We are left to conclude that in Cerebratulus dismemberment is at least greatly assisted, if it be not entirely produced, by a sudden and excessive contraction of these transverse muscles of the body. The dorso-ventral muscles being metamerically arranged, and the others being evenly distributed, dismemberment would occur at a definite place in each segment.

The final question is one which demands the method of regeneration; we shall note only the most important points. Immediately after the rupture is completed the walls of the body are drawn together, and held in that position by the contracted circular muscles. This brings the severed edges of the ectoderm into contact with each other, and they quickly unite and heal the wound over with the exception of the posterior end of the intestine, which is left open to function as an anus. The cells in this newly formed ectoderm, and the mesoderm cells immediately beneath them, proliferate rapidly and form a regenerating papilla which grows backward, keeping the anus open at its extremity. This papilla is much paler in colour than the rest of the body, and at first consists of a thin ectoderm entirely filled except at the centre with mesoderm cells. At the same time the entoderm lining the intestine grows backward as a straight tube along the centre of the papilla. The muscular system is, of course, developed from the mesoderm, and two things are worthy of note in its differentiation. The circular muscles appear first, and are followed by the outer longitudinal layer. The latter is developed very slowly, and for a long time consists of a

meshwork of longitudinal and transverse fibres which cross each other nearly at right angles. The longitudinal fibres are then increased greatly in number and size, while the transverse ones remain about the same.

But the chief interest centres about the nervous system. There has been a great deal of controversy over the origin of this system in Nemerteans. Salensky in 1884 declared, as the result of his researches on Monopora vivipara, that the nervous system was derived from the epiblast (**44**).

Hubrecht, two years later, declared that in Lineus obscurus no portion of the central nervous system takes its origin in epiblast, but that it is all of mesoblastic origin, and he undertakes to disprove Salensky's statement in regard to Monopora (**26**).

Finally, Barrois and other investigators derive certain important portions of the nervous system from invaginations of the œsophagus, i. e. from hypoblast (**7**). All these investigators were working with embryonic material, in which it is usually very difficult to determine the exact relation of the different parts. It is interesting to note in this dismembered Nemertean the mode of regenerating the lateral nerve-cords, since the process is attended by phenomena which leave no possible doubt as to the origin of the new tissue.

At the same time that the muscle-fibres first appear we find upon the ventral surface two invaginations in the epiblast (fig. 21), forming two parallel grooves running longitudinally along either side of the central line and quite near to it.

The ectoderm external to these grooves contains gland cells, that in the grooves themselves and in the space between them contains no gland cells, but seems to be made up entirely of epiblast cells. A third groove then appears in the centre of the space between the other two, dividing it into two low ridges (fig. 23). Toward the inner surface of each ridge the epiblast cells are gradually changed into neuroblasts, and form one of the long nerve-cords. These cells are not fully differentiated, but seem more like the so-

called Ersatz cells, destined to give rise to epithelium, but still capable of being transformed into neuroblasts.

After being formed in this ventral position the nerve-cords migrate upward along either side of the body to their final normal situation, carrying with them a thick covering of the neuroblast cells (fig. 22). As soon as the nerve-cords leave the ridges, gland cells begin to appear in the epiblast covering the latter, and it becomes in all respects like that on the outside of the grooves. The grooves then disappear, and leave a smooth epithelial surface.

The new nerve, therefore, is in no respect an outgrowth from the old one, but results from a new growth of cells which are entirely independent of the old nervous system.

It hardly seems reasonable that this Nemertean in its embryology should derive its nervous system from mesoblast when in regenerating lost parts it shows such a distinctively epiblastic origin.

Larval Development.

Sexual Organs.

The sexes are distinct as in most Nemerteans. There is no perceptible difference in external form or size, but in the breeding season there is a difference in colour, due to the sexual products which show through the body-wall.

The male then appears cream-coloured, while the female is greyish red, turning to a light chocolate-brown in many cases.

This difference is clearly seen in the male and female photographed in fig. 3, the latter being much the darker of the two.

In regard to the original formation of the genital pouches, nothing can be offered in the present paper, since the development is not followed that far. Hubrecht considers (26) that they arise as invaginations of the ectoderm in Lineus, but Bürger's comment seems far more probable.

He says (**13**, p. 480), "I have not been able to decide upon the origin of the sexual pouches in those forms in which they are preformed (e. g. Drepanophorus, Cerebratulus), but I am convinced that they are of mesodermal origin because they arise as clefts in the parenchyme or the dorso-ventral muscle layer, while there is no possible doubt that the sexual pouches in those forms in which they are not preformed (e. g. Carinella, Malacobdella), but are developed in conjunction with the sexual products, arise from the parenchyme, and hence are a tissue derived from the mesoderm."

The development of these pouches in a regenerating papilla bears suggestive testimony toward the same conclusion.

We have already stated that in regeneration a thin ectoderm grows backward from the surface of rupture to form the exterior of the papilla. At the same time the entoderm forms a straight tube through the centre, which is of the same size as the central lumen of the intestine since the anus remains at the extreme tip of the papilla, and which is at first without any side pouches (*en.*, fig. 60). The space between ectoderm and entoderm is filled with mesoderm, from which is developed the body musculature.

The circular muscles and the two longitudinal layers appear first, but are quickly followed by the dorso-ventral muscles.

A row of the latter muscles, each consisting of several fibres, appears on either side of the intestinal tube and close to it, at about the position they are to occupy when regeneration is completed. These form the thickened pillars at the inner ends of the germinal pouches (fig. 60, *dvm.*). Two lines of smaller vertical muscles extend from these pillars outward to the body-wall, diverging slightly. They are woven together by connective tissue so as to form walls which eventually separate germinal pouches from intestinal cæca. As the papilla increases in width the walls grow also, each pillar with its diverging walls enclosing a germinal pouch (*o.*).

As soon as the pillars and walls begin to form, the ento-

derm of the intestine grows out to the right and left between the pillars and along the side of the muscle walls, thus forming the intestinal cæca. A blood-vessel, connecting the dorsal with the lateral vessels, is formed in the connective tissue at the inner end of each cæcum (*bv.*). The genital pouches are thus bordered anteriorly and posteriorly by bands of dorso-ventral muscles, above by the proboscis sheath, and below by the lateral blood-vessel beneath the intestine.

An epithelium now appears, made up of small irregular nucleated cells which cover the pouch unevenly. It is impossible for this epithelium to be formed by an outgrowth from either the ectoderm or entoderm, since it nowhere comes in contact with either of them. It is evidently derived from the parenchymal tissue which binds together the vertical muscles to form the wall of the ovary or testis.

In winter the pouches are much smaller than the cæca, and are flattened between the latter until their sides meet.

Early in spring the sexual products begin to develop, appearing first at the outer end of the pouch nearest the muscles of the body-walls. In the figure given (fig. 63) it will be seen that the pouch extends, in the form of separate longitudinal pockets, both forward and backward along the ends of the adjacent cæca (cf. figs. 61 and 62).

Oögenesis.—The first appearance of egg development is an increase in one of the nuclei of the epithelium lining these pouches. This is carried so far that the nucleus with its large nucleolus comes to occupy a large portion of the cell.

It is then gradually surrounded by a layer of fine-grained protoplasm which protrudes into the lumen of the pouch (*ec.′*, fig. 65).

Subsequent development consists in a continued growth of both cell body and nucleus, the latter developing into the large germinal vesicle and the former spreading somewhat over the adjacent epithelium cells which have remained apparently unchanged (*ec.″*). This gives the ovum a flask shape, the neck of the flask being inserted between the epi-

thelial cells into the underlying connective tissue. As the ovum matures the neck of the flask is gradually pinched off from its connection with the epithelium, and the egg is set free in the central cavity (*ec.‴*).

It is not, however, naked as at first, but is now invested with a delicate follicle, and is soon surrounded by a thick layer of gelatine, both of which are apparently formed from adjacent epithelium cells. As fast as an ovum is pinched off into the central cavity the epithelium partly closes behind it and new ova are formed, this process continuing until the whole pouch is filled.

Interspersed among the ova and scattered through the jelly which fills the central cavity are small spherical highly pigmented bodies, granular in structure.

These are probably the same as those described by Hubrecht (27) for Drepanophorus and Cerebratulus marginatus, and like them they disappear gradually as the ova ripen. Hence they must contribute to the development of the egg, and undoubtedly furnish the yolk material. There are also other cells, slightly smaller and lighter in colour, but staining more deeply, which are scattered all through the central cavity. From these comes the gelatine which fills all the space not occupied by eggs (*gc.*, fig. 65).

In a ripe ovary the eggs are crowded together so closely that they become more or less angular in outline.

If a mass of these eggs be examined before they have touched any water they present the appearance seen in fig. 18. Each egg is separated from its fellows by the thick layer of transparent gelatine which surrounds it. This is bordered in turn by a stiffened external surface, where it comes in contact with the coats of adjacent eggs. This gives the whole mass somewhat the appearance of honeycomb, made up of angular gelatinous cells, with an egg in each cell very near its centre.

The gelatinous envelopes cling to the eggs almost as firmly as the similar jelly around amphibian eggs (Amblystoma, etc.), and they evidently serve the same purpose.

The ripe ovum is spherical, quite large (0·1 to 0·2 mm. in diameter), and appears white to the naked eye, but by transmitted light is seen to be dark brown and opaque, owing to the large number of yolk granules which it contains. It possesses a germinal vesicle fully one third its own diameter, much lighter in colour, translucent, and slightly elliptical in outline.

Inside the germinal vesicle is a large spherical nucleolus, which is nearly always eccentrically placed at one end of the vesicle, the end farthest from the original point of attachment (*ec.'''*, fig. 65).

As the eggs are developed the ovaries increase greatly in size, until finally they occupy nearly all the space, and the cæca are flattened between them.

Formation of Oviduct.—The ovaries now push inward nearly to the wall of the intestine, and outward, downward, and upward to the body-wall. In both horizontal and vertical sections they appear larger at either extremity than in the middle.

When the eggs are nearly mature each sac pushes out into the longitudinal muscle layer, presumably at the point where it meets least resistance, i. e. on the dorsal surface about one quarter of the diameter of the body from its lateral edge.

It then penetrates the muscles as a fine canal, the first beginning of a genital duct, which as soon as the eggs or sperm are fully ripe pushes through the skin to the exterior. The epithelium lining this canal is made up of flattened cells, which are elongated until they look almost like muscle-fibres.

Just inside the mouth of the duct there is a cluster of gland cells, which secrete a large amount of mucus.

As maturity advances the ducts grow shorter, in consequence of the distension of the body and the resultant decrease in thickness of the body-walls. Hence when the genital products are ripe they are easily discharged through these ducts.

As soon as the egg enters the water it is seen to be sur-

rounded by a zona pellucida, which swells up in fifteen or twenty minutes to about twice the diameter of the egg itself (fig. 20).

This zona pellucida is entirely distinct from the gelatinous envelope already mentioned, from which it is separated by two concentric membranes, situated close to each other and at quite a distance from the surface of the egg. The membranes and the zona pellucida itself are perfectly colourless and transparent. Soon after the eggs are placed in water for artificial fertilisation, or soon after they enter the water when laid naturally, the outer gelatinous envelope dissolves and disappears, leaving the egg surrounded only by the zona pellucida and the membranes. If the eggs are placed in water before they are fully ripe the outer envelope does not disappear, but remains holding the eggs together in bunches.

After the ovum is pinched off into the central cavity of the ovary it retains its original flask shape for a long time, sometimes even after it is laid. Hence it occasionally happens that after the outer gelatinous envelope has disappeared the limiting membranes will be found to possess a teat-like protuberance on one side, which is manifestly the remains of the original connecting stalk (fig. 20). And sometimes a corresponding remnant may be found upon the egg itself, the neck of the old flask which has not been wholly withdrawn into its body. These protuberances are of special significance, because they enable us to orient the eggs perfectly, and to determine that the polar bodies always come off at a point diametrically opposite to them, and hence opposite to the original point of attachment. It should be added that since the egg is always elongated into its flask shape at approximately right angles to the surface of the epithelium, it follows that the egg axis bears no definite relation to the axis of the mother, but may stand at any angle with it.

S p e r m a t o g e n e s i s.—The testes are formed and developed in the same way as the ovaries. The origin of the spermatozoa in Lineus has been admirably worked out by Lee (**30**). He

found that they were not developed in preformed sacs, but that they themselves gave rise to the latter, or rather furnished the occasion for their existence. A few large cells appeared in the body parenchyma, which were soon gathered into bunches, around which was developed a thin membrane, thus forming a testis. But in Cerebratulus the spermatozoa as well as the eggs are developed from the epithelium lining the genital sacs. The cells of this epithelium increase rapidly by division, and the original nuclei become larger, as in the development of the ova. Through the consequent crowding of the cells some of them are pushed out into the central cavity, where they become sperm mother-cells and form spermatozoa by segmentation (fig. 66). The transition from the sperm mother-cell, or spermatid, into the spermatozoon is apparently not direct, there being intermediate stages (fig. 68).

But the chromatic substance of the nucleus of the spermatid gradually separates itself as the head of the sperm, while the body draws itself out into a long flagellum (cf. figs. 67 and 68). The general type of development is thus very similar to that given by Lee (loc. cit.), the essential difference being that in Cerebratulus the testis is preformed and the sperms are developed from its epithelium.

When fully ripe the sperms are very large (0·05 mm. long) and of a peculiar shape (fig. 59; compare also fig. 42, in which a sperm and the polar bodies are drawn to the same scale).

The head is long and sickle-shaped, tapering to a very fine point at the anterior end, and usually somewhat curved into a crescent. The middle piece is nearly spherical, and caps the posterior end of the head like the top of a clothes-pin.

The tail or flagellum is very long, many times the length of the head and middle piece, and is undivided.

These sperms have a slow movement, but it is seemingly very strong, for they experience no difficulty in getting through the two egg membranes in order to reach the egg itself.

Egg-laying.

The breeding season extends over about two months or ten weeks. Some of the females begin laying by the 10th of June, while others delay till the 20th or 25th of August. In general those which live in sheltered coves or bays lay earlier than those found in exposed positions, while the nearer low water mark the worm is found the later will its eggs be deposited. By changing the locality, therefore, from which the eggs are obtained, fertile ones can be secured throughout the entire two months. Although Casco Bay is but little north of Long Island Sound, the above dates are radically different from those given by Coe (**15**), who states that in the vicinity of New Haven the breeding season extends over the month of April, and that by "the first of May nearly all the genital products have been discharged." This serves to emphasise still farther the marked differences which have been noted in the breeding seasons of other Invertebrates from these two localities.

Bürger (**13**) states that Nemertean eggs are usually joined together in strings or balls, but that they are rarely laid singly. Metschnikoff (**35**) gives Lineus lacteus as one of these rare instances, but he tells us nothing whatever as to the manner in which they are laid.

From the rapidity with which the outer gelatinous envelope disappears when the eggs are placed in water for artificial fertilisation, it would be naturally inferred that the eggs, if not laid singly, at least separated very quickly, since this envelope would be all that held them together. That this is what actually occurs was proven by observation. The first lot of eggs laid in the aquarium (cf. p. 113) were found early in the morning already fertilised. A day or two later the process was repeated while the aquarium was being watched, also early in the morning. A stream of eggs was noticed coming out of the opening of one burrow, while from another burrow at the opposite side of the aquarium sperms were issuing.

Fortunately the burrow occupied by the female was in contact with the side of the aquarium, and the whole process could be watched through the glass. It was seen that the contents of a very limited number of genital pouches near the anterior end of the body were being emptied. The eggs were assisted in their passage through the oviduct by a contraction of the circular muscles of the body-walls over the area occupied by the pouches. When first issuing from the body the eggs were gathered in loose bunches, held together by the gelatinous tissue already described. These bunches were pushed along gradually by successive waves of muscular contraction in much the same way that the ring of mucus formed by the earthworm in egg-laying is worked off the forward end of its body.

In this way the eggs came to lie at the bottom of the vertical portion of the burrow around the head of the Nemertean. Here the action of the water quickly dissolved the gelatinous material that held them together, and left them free. They were then caught in the current generated by the Nemertean's breathing, and carried upward out of the mouth of the burrow and some distance away from it. Eggs were laid in this manner several times, and upon them were verified all the phenomena of maturation and segmentation derived from artificially fertilised eggs. But in so restricted a space there was always a tendency to over-fertilisation, and many of the eggs were killed in this way. Whenever a considerable number were laid at once they collected together at the bottom of the aquarium, and were held together loosely by adhesion of the zonæ pellucidæ, but a slight disturbance in the water separated them and they floated away free.

Nothing could be seen of the burrow occupied by the male except its opening, but as sperms were issuing from this in the same way that eggs were from the other, and as a thick mass of sperms was afterward found at the bottom of the burrow in a position corresponding to that of the eggs, it would seem safe to conclude that the sperms are discharged in the same way as the eggs.

It follows that the eggs must be fertilised outside the body of the female, and that the sperms often have a considerable distance to travel before reaching the eggs. This will account in part for their very strong motion, and it also verifies the statement made by Coe (**15**) that all the genital products are not discharged at once, but only a limited portion of them. From the fact that the several lots of eggs were laid so near together, it seems probable that the period of egg-laying in any one individual does not occupy more than a week or ten days. When Nemerteans are kept in confinement without any chance to burrow, they usually pull themselves in pieces after a short time. If the sexual products are ripe when this occurs they are always discharged, and perfectly healthy fertilised eggs may often be obtained in this way.

Darwin, in his 'Descent of Man,' says that these animals, like many other Invertebrates, "apparently stand too low in the scale for the individuals of either sex to exert any choice in selecting a partner, or for the individuals of the same sex to struggle in rivalry." This is undoubtedly true, but we must remember with McIntosh that this does not exclude the possibility of a very delicate sexual instinct. The simple fact that the sperms are discharged simultaneously with the eggs, even though the burrows of the male and female may be some distance apart, proves the existence of such an instinct.

Fertilisation and Ripening.

There is nothing resembling copulation, eggs and sperm being discharged from separate burrows. When ripe the eggs fertilise almost immediately, but with a little care the entrance of the sperm into the egg may be seen, though its subsequent action must remain invisible owing to the opacity of the yolk granules. A great many sperms usually penetrate the limiting membranes, but only one of them enters the egg, though instances of polyspermy are not unknown. The sharp curved point of the sperm is placed against the surface of the egg, and a gentle vibratory movement is pro-

duced by the lashing of the long tail. Although this appears like a weak movement under the microscope, it must really be very strong, for the head of the sperm is pushed in steadily and quickly.

The curve in the head of the sperm renders any rotary motion impossible. As soon as it has entered the egg the latter becomes more opaque than before, and the large translucent germinal vesicle quickly breaks down and disappears. This is followed in turn by the giving off of the polar bodies.

If the supply of sperm be sufficient, so many of them penetrate the membranes that the latter are broken down and disappear entirely. Then the sperms, clinging to the egg with their heads and lashing with their tails, give the egg a rotary motion, exactly similar to that produced in later development by the cilia. This may be kept up for an hour or more.

Formation of Polar Bodies.—The first polar body appears about an hour and a quarter after fertilisation, and always at a point diametrically opposite the protuberance in the limiting membrane, when such a protuberance is present.

As already noted, this is the remnant of the original stalk whereby the ovum was joined to the wall of the ovary, and hence those eggs which do not exhibit it must have been separated so gradually and completely as to have left no trace of their former connection. But it is reasonable to infer that the polar bodies appear in them also opposite to the point where such connection formerly existed. If this be true, it follows that the connection of the ovum with the wall of the ovary during development results in its permanent polarisation, and the point at which the polar bodies are to appear is predetermined.

There is no perceptible separation of formative and nutritive material, but correlated phenomena indicate that the point of attachment is the vegetative pole. The food supply must come to the ovum through this attachment, and in early development the germinal vesicle often lies eccentrically near it (fig. 64). These facts, taken in connection with what has

already been given in reference to the early development of the ovum, furnish strong corroborative evidence to the truth of the view formulated by Mark in 1881 (**32**) and by Watasé ten years later (**47**). The ovum is at first isotropic, but afterward acquires polarity and other promorphological features through its topographical relation to the remaining cells of the maternal tissue. In this species the primary axis of the ovum seems to be formed at right angles to the surface of the epithelium from which it originates (cf. **50**).

The appearance of the first polar body is accompanied by the usual flattening of the surface, and sometimes by a slight pitting in of the ectosarc to meet it (fig. 23*a*). The latter effect is often abnormally increased if the cover-glass is allowed to press upon the egg. The gradual emergence and rounding of the first polar body is shown in figs. 24—26.

A slight swelling first appears at the point of emergence, and rises as a symmetrical hemisphere. This increases in size, and within a minute becomes a short cylinder, or a more or less rounded conical protuberance (fig. 24).

Within half a minute more the base of the cylinder is constricted, and the constriction increases until the polar body has entirely separated from the egg (fig. 26). So far as observed a connecting protoplasmic band is always present, but it requires a good light and a high power to detect it.

The flattening of the pole draws the surface of the egg away from the membranes, so that the polar body after its extrusion is not very near them. It now becomes approximately spherical, the whole formation having occupied about three minutes.

It lies in contact with the surface of the egg or near it for ten or fifteen minutes, during which the egg resumes its original shape, and then withdraws a little, but still retains the connecting band of protoplasm. This withdrawal is simultaneous with the first appearance of visible spin-threads, and marks the beginning of a long series of remarkable activities which are of sufficient importance to demand a separate description (see p. 133).

About six or eight minutes later the first body returns to a position near the egg, and there awaits the second body.

A swelling appears at the point where the connecting band of protoplasm from the first body joins the egg (fig. 28). This rapidly becomes nipple-shaped, lifting the first body upon its slightly enlarged apex (fig. 29). There is the same gradual constriction as before, and the whole formation occupies about the same time, three minutes. There is also a second flattening of the egg, sometimes a slight emargination, whereby the surface is withdrawn from the membranes, so that the combined first and second bodies, even though the latter is elongated, do not get any nearer to them. The second body is smaller than the first, and does not become spherical, but is elongated parallel to the surface of the egg into a spindle or melon shape (fig. 35).

Sometimes the first body divides after separation, or, instead of a single second body, two occasionally appear side by side.

In both these abnormal cases one of the paired bodies withdraws by itself, while the other two remain and continue their filose activities.

Activities of the Polar Bodies.—During their formation and almost to the blastula stage the polar bodies afford a remarkable example of that form of protoplasmic activity which was first described in echinoderm eggs by G. F. Andrews, and designated "filose activities or spinning" (4).

These activities have already been carefully described in this Nemertean in an interesting paper by Dr. E. A. Andrews (2), in which some doubt was expressed as to the certainty that such activities were entirely normal. The eggs used by Dr. Andrews were fertilised artificially, but great care was used in observing them, and the results were verified by comparison with other eggs which developed into normal embryos. I am glad to add in confirmation the following description taken from eggs laid in an aquarium under fairly normal conditions and fertilised naturally.

The first polar body, immediately after separation, exhibits amœboid changes of outline, as can be seen by comparing different figures. These are most prominent during the first four or five minutes. At the end of this time the body has become spherical, and stands close to the surface of the egg, with which it is connected by a narrow band of protoplasm (fig. 26).

It now begins to exhibit spinning activities. Numerous fine transparent threads of protoplasm radiate outward from its surface, usually appearing first at one end (the body is considered as lying with its side toward the egg), but frequently elsewhere. The whole surface becomes covered with such radiating threads, which vary considerably in size and visible length. At the same time the body moves away from the egg slowly, but whether as a result of the spinning or the amœboid movement or both could not be determined.

No further change occurs until the appearance of the second polar body, except that sometimes the surface of the egg in the vicinity of the first body rises up into one or more small papillæ. These occur most frequently on eggs which afterward become abnormal, and they disappear quickly.

As soon as the second body appears there is a distinct change in the activities of the first body. The body itself is slightly elongated parallel with the surface of the egg, and becomes flattened on the inner side. The filose threads, which have been hitherto distributed over its surface, now aggregate toward one or both ends, and gather into long filaments visible under a low power. They extend outward and downward toward the surface of the egg (fig. 31), but could not be traced to actual contact with the surface. But this affords no proof that they do not reach it, for they are extremely delicate and transparent, and they diminish in size the farther they are followed, so that it becomes difficult to see them.

Certain phenomena at just the place on the egg toward which they point indicate that they really do reach the surface.

Furthermore, as Dr. Andrews has noted (2), the appearance of the first body during the extrusion of the second, and its subsequent changes in shape, are just what they would be if the first body were pushed upward by the second and at the same time held down to the surface of the egg by these filaments.

The band of protoplasm connecting the first body with the egg remains after the second body is formed, and connects the two bodies. The second body immediately begins to elongate parallel with the surface of the egg, and assumes a spindle or melon shape, which always characterises it. A small papilla now appears on the egg at either side of the second body and some little distance from it (fig. 33). These two papillæ are situated exactly where the long filaments from the first body would strike the egg if they reached that far, and it seems probable that the two are connected. The papillæ vary in size and number on different eggs, but they are usually two.

They are arranged symmetrically, one upon each of what will soon become the first two blastomeres (fig. 34). It is also significant that the spindle of the egg nucleus after the extrusion of the first polar body rotates through 90°, as in so many other eggs, and becomes parallel with the surface, its two ends being just beneath the points where the papillæ afterward appear (fig. 56).

The summits of the papillæ are covered with fine spin-threads, radiating outward, while the surface of the egg between them is flattened or even concave. They continue for about five minutes and then disappear (fig. 36). In eggs not quite ripe, or developed under abnormal conditions, especially pressure, the papillæ are increased in number, size, and duration (fig. 37).

I have repeatedly seen unripe eggs, on reaching this period in their development, put out ten or twelve large pseudopodia-like processes in place of the two papillæ, and gradually go to pieces.

When the papillæ disappear the egg returns to its

original form, thereby carrying the polar bodies nearer the membranes.

In one or two instances the recovery was carried so far that the polar bodies were pushed out into the membranes as in fig. 39.

This figure shows that there are two independent membranes, for only the inner one is bulged outward to any extent.

It also shows that the first polar body is surrounded by a zone within which spinning activities are sufficiently strong to prevent the membrane from entering. The relative distance of the two bodies from each other and from the egg does not seem affected at all by this contact with the membrane, so that the latter must be very delicate and yield readily to slight pressure. The second body in pinching off from the egg also leaves a narrow band of connecting protoplasm (fig. 35), which keeps both bodies in close relation to the egg.

When separated it begins to show activities somewhat different from those of the first body (fig. 36). While forming, fine threads are seen radiating outward from nearly all visible parts of its surface, but as soon as it assumes its characteristic spindle shape the two poles at the extremities of the spindle become the centres of filose activity.

At first they send out slender threads in various directions, but soon they begin to elongate as pseudopodia processes (fig. 35), carrying the centre of activity away from the body itself. The ends of the processes enlarge, and may even form secondary spindles, from which fine threads radiate in all directions (fig. 38). Short filaments still appear and disappear at different points on the body itself, but they are very transitory, and evidently of secondary importance.

In unripe eggs, and especially in those developed under abnormal conditions, the spinning activities may be greatly increased. These are the eggs usually covered with papillæ in the vicinity of the polar bodies, and the abnormal spin-

threads run back and forth between bodies and papillæ, lacing them together firmly (fig. 37). But even then the threads are remarkably independent of one another, and in no instance were two of them seen to fuse where they crossed, though sometimes one would divide and attach itself by two or more strands.

These abnormal activities also emphasise the fact that those spin-threads which connect polar bodies and egg emanate from the former. Of the spin-threads arising from the egg the longer ones do not extend toward the polar bodies, while the shorter ones, which do, evidently do not reach across.

The polar bodies take the initiative in spinning; and, although the egg responds, it never reaches the same degree of activity.

If a higher power is used the polar bodies are transparent enough to disclose something of their internal structure.

The first body is pear-shaped, with a short process extending from the smaller end like a stem (fig. 41). Numerous threads radiate in every direction from the enlarged tip of this process, while a similar bunch of threads radiate immediately from the body at its opposite end, and a few weak threads appear on the outer side. The interior is filled with fine-grained protoplasm, with chromosomes at or near the centre.

The normal number of these seems to be five, but they vary considerably, now appearing as five distinct curved rods (fig. 41), and again fusing to one large mass, with or without a smaller one beside it (fig. 40). The same chromosomes may be seen in the second body, sometimes separate (fig. 44), at others more or less fused (fig. 45). The fusion in both bodies occurs at the time when they frequently divide, but the karyokinesis does not show very plainly. During the emergence of the second body the first one shows quite a marked polarisation (fig. 34), but the resultant figure is never as perfect a spindle as in the second body, and the polarisation disappears early in segmentation (fig. 44). The second body, on the contrary, maintains its characteristic spindle shape through-

out segmentation, its ends are enlarged more than those of the first body, and they continue to be centres of filose activity nearly to the blastula stage (cf. figs. 44, 45).

Summary.—1. The first polar body acquires its spinning power soon after separation, manifests it chiefly in the form of diffused spinning with little polarisation, and loses it early in segmentation. The second body acquires this power during separation or immediately afterwards, possesses a marked polarisation, and retains its activity at least to the blastula stage.

2. In both bodies the poles are at either extremity of an axis parallel to the surface of the egg, and the spinning activities are more or less centred there. In consequence these two points assume a character notably similar to that of the two centrospheres at either end of the nucleus during segmentation.

3. Both bodies show in their interior chromosomes, whose normal number seems to be five, but which vary greatly in number as the result of partial fusion.

4. The surface of the egg in the vicinity of the polar bodies also exhibits spinning activities during and subsequent to the formation of the bodies. This spinning is quite diffuse, but just after the separation of the second body it becomes concentrated at two papillæ symmetrically arranged, one on either side of the first axis of segmentation. The long spin-threads from either end of the first body connect with these papillæ. The internal phenomena of fertilisation and segmentation are invisible through the yolk granules, but may be seen in sections.

Conclusions.—1. The polar bodies and the egg itself, in this species, are to a large extent physiological as well as morphological equivalents.

Evidently the half of the original egg nucleus which remains within the egg retains the same characteristics as the halves which are successively extruded in the polar bodies, or else the latter acquire distinct characteristics during extrusion—an hypothesis which is hardly tenable.

Hence the polar bodies on the exterior, where they are plainly visible, repeat for us the same activities, or very similar ones, that occur invisibly within the egg. We find both bodies elongated at right angles to the first plane of cleavage, while their ends, enlarged over each blastomere, become centres of spinning activity.

Externally the surface of each blastomere also manifests spinning activity by rising into a papilla which becomes connected with the enlarged end of the first body on its own side.

These visible filose activities of the egg are much more prominent while the egg nucleus remains near the superior pole, and diminish sensibly as it descends to meet the sperm nucleus.

Such a result would naturally follow the intervention of inert yolk material between the active nucleus and the surface of the egg. The increased activities of some eggs immediately after the extrusion of the second polar body (**2**, p. 232, and fig. 37), whereby the whole surface of the egg at the superior pole is raised into ridges and papillæ covered with filaments, admit of ready explanation if we assume that in such eggs for some reason the nucleus does not descend at once, but tarries awhile close to the surface.

Internally the female pronucleus, after the extrusion of the first polar body, rotates through 90°, as already stated, so that its ends are just beneath the points where the papillæ appear (fig. 56).

This has been noted in Cerebratulus marginatus also (**16**, figs. 20 and 22), where the amphiaster is plainly parallel to the elongated polar body. Furthermore there is good evidence for believing that the female pronucleus again rotates after the extrusion of the second body, and becomes parallel with the latter (fig. 57). But with the amount of material at my command for this particular phase of development I have been unable to decide beyond a doubt. After the egg nucleus has united with the sperm nucleus the resultant segmentation nucleus is also elongated at right

angles to the first plane of cleavage, and forms an amphiaster. Its ends are not enlarged in each blastomere, but there is instead a centrosome at either end, which becomes the centre of activities very similar to those manifested by the enlarged ends of the polar bodies (fig. 58).

Is it too much to conclude that this marked similarity between external and internal phenomena cannot be the result of chance, but that there must be a close relation between the two? These considerations lead naturally to our second and third conclusions.

2. In Cerebratulus we have an instance where oögenesis approaches more closely to spermatogenesis, and thereby shows more clearly the morphological equivalence of the two.

Adopting the view first put forward by Mark in 1881 (**32**), that the polar bodies are to be regarded as abortive eggs, we find in Cerebratulus an instance where they are less abortive than usual. In spermatogenesis the spermatocyte usually divides into four sperms, each of which is in every way the equal of all the others. But in the final division of the oöcyte the perfect egg ordinarily appropriates to itself all the activities of the three rudimentary ones, as well as the entire mass of the yolk. In Cerebratulus, however, it contents itself with taking all the yolk, and leaves quite a share of the activities for the polar bodies, especially the second one.

3. The egg-cytoplasm, and probably the female pronucleus also, exhibit a well-marked polarity at right angles to the first plane of cleavage before the two pronuclei have joined to form the segmentation nucleus.

This can be easily seen by reference to figs. 34 and 56. Even in those exceptional instances where the whole polar surface is covered with ridges and papillæ the same transverse polarisation is manifest in the increased size of certain of the papillæ (fig. 37; cf. also **2**, fig. 12).

Segmentation.

Segmentation is total and equal. The first furrow lies along an axis joining the polar bodies and the point of original attachment. This fact furnishes further evidence that the axis of segmentation was determined early in development. About an hour and a quarter after fertilisation the egg begins to elongate at right angles to the axis just mentioned, thereby drawing the polar bodies away from the membranes. It continues to elongate until the long diameter becomes one and a half times the shorter one.

If viewed at this period under a low power with strong illumination, it can be seen that the nucleus has divided, and the halves have migrated to either end of the ellipse.

A shallow groove appears beneath the polar bodies and the egg elongates still more, the diameters bearing the relation to each other of five to eight. Two minutes later a corresponding groove appears on the opposite side, and they both deepen rapidly, the first more rapidly than the second. There is also a continued elongation, the diameters standing in the relation of five to nine. At the end of this period, which has lasted ten minutes, we find the egg divided into two almost perfectly spherical blastomeres, joined by a very narrow isthmus, whose distance from the polar bodies is twice that from the opposite pole (fig. 49).

Behaviour of the Polar Bodies.—Usually the flattening of the egg preliminary to segmentation moves the polar bodies, but sometimes the connecting band of protoplasm elongates and leaves them in their original position. When the furrow appears they are always pulled down into it. Under a low power it is seen that they follow the changes in position which the surface undergoes as if they were attached to it.

Under higher magnification one can detect that the bodies themselves are somewhat changed in contour by the process (fig. 42). Instead of its characteristic spindle shape, with the long diameter parallel to the surface of the egg, the

second body is pulled out at right angles to that surface, until sometimes the perpendicular axis becomes the longer one. Evidently its attachment must be firm enough to bring about such a change.

Its spinning activities are not disturbed in the least, and the former poles remain the centres of such activities. This often results in an irregular quadripolar appearance, the two connecting bands of protoplasm arising from the vertical poles, while from the horizontal ones radiate long filose spin-threads.

We have said that the cleavage groove nearest the polar bodies usually occupies two thirds or more of the entire diameter of the egg. Hence, if the polar bodies remained as near the bottom of the groove as they were when it started, they would be drawn in at least to the centre of the egg. But such is not the case. They do sometimes enter within the general contour of the whole mass, but they usually follow the groove only a little way, and remain partially or wholly outside the general contour. As a result, the band of protoplasm connecting the second body with the egg apparently changes its point of attachment to the latter. Instead of remaining near the bottom of the groove, it removes to a comparatively long distance from it on the side of one of the blastomeres (fig. 46).

Is this change of position real or apparent? Is it the body which has changed, or the bottom of the groove?

That portion of the egg where the connecting band originally appears is the centre of activity during the extrusion of the bodies, and naturally remains connected with them during their filose spinning. It then becomes the point at which the protoplasmic activities resulting in the first cleavage are manifested. Hence, if the point of attachment were to be changed, it would seem natural that it should be toward some other centre of activity. But no such centre can be found at or near the surface of either blastomere, so that a priori we should not expect any change. Again, the only way in which such a connecting band could change its position would

be either by the withdrawal of the filose threads of which it was composed and the protrusion of others from a new point, or by a gradual slipping along the surface. The latter hypothesis is untenable from the very nature of the spin-threads, and the most persistent watching failed to reveal any temporary withdrawal of the connecting protoplasm. We are left, therefore, to the conclusion that the change is only apparent, and that the actual point of attachment remains the same. This affords a means of identification whereby we can follow one particular portion of the surface during segmentation. In this way we find, first, that there is no investing membrane in contact with the surface of the egg, but that the protoplasm of the latter forms an ectosarc similar to that of the amœba. This is lighter in colour and more transparent than the remainder of the egg, partly because it contains no yolk granules.

Again, we find that the change of position is due to the fact that the point of attachment remains stationary, while the groove advances; in other words, the old ectosarc travels inward, during the formation of the groove, only the limited distance necessary to accommodate the more rounded outline of the blastomeres, and the ectosarc which covers the bulk of the groove is a new structure. This is easily formed, since it consists essentially of egg protoplasm *minus* its yolk granules.

Anticipating a little, we find that after the two blastomeres have come together again, and the furrow has been filled in by appression, the point of attachment does not return to the bottom of the furrow, but, approaching it closely, remains at a distance which varies within narrow limits for different eggs (fig. 50).

The two edges of the old ectosarc do not quite meet, but a portion of the new ectosarc remains exposed along either side of the furrow. This portion increases with subsequent segmentations until the point of attachment comes to lie near the centre of the cell in the 128-cell stage (fig. 45).

This is the natural result, since the egg increases slightly in size with each segmentation. The protruding surface of

each blastomere retains a portion of the old ectosarc near its centre, and this is surrounded by a border of newly formed ectosarc, whose relative width increases as segmentation advances.

Finally, the persistence of the point of attachment of the second polar body greatly facilitates the orientation of the egg. As soon as the first groove is formed the blastomeres become almost perfectly spherical. Most eggs having total cleavage show a rounding of the blastomeres, but in this Nemertean they are almost separated, so that the egg assumes a dumb-bell shape, with a short handle eccentrically placed.

As would be inferred, they are easily separated, with results to be noticed later.

Certain interesting phenomena precede the coming together and flattening of the blastomeres. In this egg, as in that of Echinoderms (**5**), filose activities similar to those of the polar bodies arise on the surface of the blastomere.

In fact, they are continuations of the same activity which has just been spinning the connecting bands of protoplasm and producing the first cleavage groove. Fastened at a point near the opening of the groove are the polar bodies (fig. 46).

Close watching in a good light now reveals spin-threads forming between the blastomeres. A small papilla appears nearly opposite the attachment of the polar bodies, and gradually increases until it becomes half as large as one of them.

At first filose threads radiate from this papilla in different directions, but they gradually fuse into one thick thread, extending obliquely across the groove to a point a little below where the polar bodies are attached (fig. 47). Half a minute later another spin-thread appeared below the first, but no papilla could be seen at its origin, probably because it was not in perspective. This thread divided when half-way across, and the two ends were attached separately (fig. 47).

At the same time spinning activities began on the opposite

side of the connecting isthmus. A large papilla was formed on the same blastomere to which the polar bodies were attached, and a moment later a similar one appeared on the other blastomere (fig. 47). Short spin-threads radiated from both papillæ; then the first papilla disappeared, but the spin-threads remained and formed quite a stout thread extending across the groove (fig. 48). In some eggs the threads first formed remain until those upon the opposite side of the isthmus have appeared. These eggs are of special interest, because they show that the spin-threads contain contractile protoplasm (fig. 51). The two blastomeres were drawn together on the side of the isthmus farthest from the polar bodies, and held for an instant and then released. During this contraction the spin-threads shortened and thickened visibly, showing that they were at least active agents in the process. The papillæ also were pulled out slightly in the direction of the spin-threads, as would be expected of yielding protoplasmic material. After an interval of five seconds the blastomeres were pulled together again, this time on the side nearest the polar bodies. These movements on opposite sides of the isthmus alternated at irregular intervals for two minutes, when the papillæ had entirely disappeared, and the blastomeres had been drawn together and flattened. These contractile movements were noted independently, and commented upon by a friend who was watching another lot of eggs, and were observed several times subsequently while studying other phenomena, so there can be no doubt of their reality. It must be remembered in judging these facts that the conditions are specially favourable in the present instance for the occurrence of just such phenomena. Intercellular connections by means of spin-threads have been clearly demonstrated in the eggs of many Metazoa (cf. **3**), and have even been retained in preserved specimens.

We should expect to find them in Cerebratulus as a natural sequence to the marked spinning activities of the polar bodies. The perceptible motion of the blastomeres is rendered possible

by their almost complete separation ; the isthmus joining them is very slender, and can be bent by a slight force.

We thus have in these eggs a combination of strong spinning activities with just the physical conditions best adapted for rendering them manifest. These activities are more profuse at this early stage than in any later period of development. Whether the blastomeres are sensibly reduced by this spinning is difficult to determine, because they are continually changing their shape at just the time when the spinning is most profuse. In immature and abnormal eggs, however, they often produce a perceptible diminution of the blastomeres, and in such cases segmentation is usually unequal instead of equal, the third furrow producing four micromeres and four macromeres. Subsequent division proceeds on the same plan, but the eggs are so weakened that they seldom reach gastrulation. In this egg, as in the Echinoderm, there is thus a marked difference between normal and abnormal filose phenomena, as emphasised by G. F. Andrews (**5**). Such abnormalities are of little use in explaining normal cleavage, but may contain valuable suggestions. Finally, we may note that these abnormal activities are frequently carried so far that the egg spins out the entire substance of the blastomeres, and goes to pieces during the first segmentation.

This first cleavage, therefore, occupies about fifteen minutes, and is followed by an interval of rest lasting ten or twelve minutes.

This interval is occupied in the various phases of karyokinesis; the nuclei of the two blastomeres divide, the halves separate and take a position at either end of the vertical diameter of each blastomere, where they can be seen under a low power.

The second cleavage groove then appears at right angles to the first, and divides the egg horizontally into four blastomeres. These are not spherical, although they appear so when viewed from the side (fig. 52). If the egg be rolled between cover-glass and slide, the blastomeres are seen to be

really egg-shaped or ellipsoidal masses lying side by side, with the polar bodies still attached to the first cleavage groove (fig. 53). When they flatten together an open space is often left in the centre as in the Echinus egg (fig. 52).

If the surface of the blastomeres bordering this opening be examined under a high power considerable filose activity is revealed. One spin-thread starts from a papilla on the side of a blastomere, and runs diagonally across to an adjacent blastomere. Several other fine threads can be seen radiating from the summit of the papilla, but they could not be followed across (fig. 52). Another thread crosses from one blastomere to the one opposite it, and makes a sharp bend or elbow halfway across, very similar to the bends figured for the star-fish blastula and the Echinus four-cell stage in the paper just referred to (**5**). The third thread visible at this level proceeded out a little way from the remaining blastomere, and then turned abruptly and ran down diagonally to an adjacent blastomere.

This central open space is subsequently obliterated by the flattening of the blastomeres. The polar bodies continue their filose activity, remaining attached to one side of the first furrow. In fig. 43 both bodies are seen to be bipolar, but the two poles of the first body are unlike.

One consists of a large but short and blunt process from whose tip and sides fine threads are given off, while the other is made up of a simple bunch of radiating threads, each of which starts from the body itself. Another blunt process projects toward the second polar body, and forms part of the connecting band between the two. Short fine threads also radiate from this process.

The second segmentation occupies not more than eight or ten minutes, and is followed by a rest of five minutes.

The third segmentation is vertical, and divides the egg into eight equal blastomeres. Subsequent segmentations take place more slowly than the first two, and the blastula stage is reached in about fifteen hours. During the second and third segmentations the egg elongates again, each time at right

angles to the plane of cleavage, and since the first three planes are in the three dimensions of space the successive elongations restore the sphericity of the egg. During the flattening together at the close of the third segmentation there is a twisting to one side, whereby the four upper blastomeres come to lie over the grooves between the lower four, i. e. the cleavage is spiral (fig. 54).

A change now takes place in the relative activity of the polar bodies; the first one has been more active hitherto, but it now loses its marked polarity and begins to resume its original spherical form. One of its poles has already been replaced by a bunch of radiating filose threads, and the other one now disappears gradually until the only remaining signs of activity are occasional spinnings from various points on the surface.

The second body, on the contrary, increases in activity; the processes at either end enlarge, and the filose spinning increases proportionally. The band of protoplasm connecting it with the egg changes continually in size and contour, and occasionally a lump or swelling appears near the centre of this band, looking like a third polar body. But it soon disappears. The two figures given (44, 45) show the relative activities in late stages of cleavage, and they also show that the chromatic substance is affected by these activities.

In the first figure the chromatin appears in the form of grains scattered through the body; in the second figure it has been gathered into a nuclear-like mass near the centre, whose size and shape change under the influence of the spinning.

This change of the chromatic substance under the influence of filose activity in the polar bodies corresponds well with the changes in position, size, and shape of the chromosomes in the nucleus during karyokinesis under the influence of the spindle activities, and is another evidence of the close relation between the two. We have no means of knowing how far they may agree in detail, but they are worthy of note as showing that the filose activities have a tendency in the same direction as those of the segmentation spindle.

A segmentation cavity appears very early, but not quite in the eight-cell stage as described by Barrois for Lineus Gesserensis (7).

As would be readily inferred, the first two blastomeres are easily shaken apart when joined only by the narrow isthmus, and the same is true of the four- and eight-cell stages.

The experiments of Morgan and others upon frogs' eggs at once suggested that blastomeres separated in this way might develop into larvæ. Accordingly experiments were made with the most satisfactory results. Even if the egg-membranes were broken in the shaking and the blastomeres set free, they segmented as though nothing had occurred, and developed into larvæ similar in all respects to those reared normally, except that they were only half as large, or even smaller in the case of isolated blastomeres of the four- and eight-cell stages.

Summary.—1. The axis of first segmentation is predetermined in the ovary at a very early stage of development. It extends from the point where the egg-cell is originally attached to the wall of the ovary, diametrically across the egg to a point where the polar bodies subsequently appear.

2. The ripe ovum is spherical. Segmentation is total and equal; the first three cleavage planes occupy the three dimensions of space, the second being horizontal. The first segmentation occupies fifteen minutes, and is followed by a period of rest lasting ten or twelve minutes. The second occupies not more than ten minutes, followed by a rest of five minutes.

Subsequent divisions proceed regularly but more slowly, and the blastula stage is reached at the end of the fifteenth hour.

3. The polar bodies remain attached to each other and to the egg through segmentation, and continue their filose activities.

All evidence indicates that the point at which the second body is attached to the egg remains constant. It appears at the bottom of the first cleavage groove, then changes, as the

groove deepens, to the side of that blastomere which first passes into the four-cell stage. After the blastomeres have been appressed it remains a short distance from the bottom of the groove. This distance increases with subsequent segmentations, until at the close of segmentation it appears near the centre of the external surface of one of the cells. These changes of position without change of identity enable us to determine—

a. That the egg possesses no surface membrane, but has an ectosarc similar to that of the amœba.

b. That the original ectosarc contributes but slightly to the covering of the cleavage grooves, the larger portion of that covering being a new structure.

c. That after appression a portion of this new ectosarc remains exposed along the lines of cleavage, and increases in relative width as segmentation progresses.

d. That gastrulation begins at the inferior pole, and the axis of first cleavage becomes the future dorso-ventral axis of the pilidium, and later of the adult (**13**).

4. The blastomeres show vigorous spinning activities during early segmentation. While the polar bodies are coming off, and immediately after the separation of the second one, numerous spin-threads appear on the surface of the egg just beneath the bodies. A papilla is formed on either side of the first cleavage plane, and from its summit spin-threads radiate outwards, some of them connecting with those from the poles of the first polar body, and re-establishing vital connection between the two. Both papillæ and spin-threads disappear before segmentation begins.

5. Papillæ are also formed on the sides of the cleavage grooves, from which spin-threads extend to adjacent blastomeres and aid in appression. These are easily seen in the first groove, less easily in the second, and can hardly be distinguished in the eight-cell stage. These threads are contractile, and during the first cleavage, when the blastomeres are almost separated, their contraction produces a perceptible movement.

6. The blastomeres may be entirely separated during early segmentation, with the result that each one produces a perfect undersized pilidium.

Conclusions.—Recent investigations (3 and 5) have disclosed in living eggs of Echinoderms, Annelids, and Molluscs activities similar to those just described, and filaments which were probably of the same nature were found in preserved eggs of Amphioxus and the Amphibia. The first observer of these activities (5) interpreted them as establishing the physiological continuity of the blastomeres during cleavage, and all subsequent investigations have only emphasised this inference.

Our first conclusion, therefore, reiterates the same truth :

1. The spin-threads connect the blastomeres and prevent any physiological separation during segmentation.

Few eggs undergo more complete separation of the blastomeres than those of Cerebratulus, but when most distinctly separated they retain their physiological unity by means of numerous cytoplasmic commissures. Evidently these spin-threads are portions of the internal protoplasm which have flowed out to the exterior, and are formed in the same way as the filose pseudopodia of Radiolarians and the larger pseudopodia of amœbæ.

We should expect to find them, therefore, as suggested (3), "high roads for the passage of living substance" between the blastomeres, and between them and the polar bodies. And under specially favourable circumstances the actual movement of granules along certain threads may be seen. The current runs now outward and again inward, as in a Radiolarian's pseudopod.

Hence in those filaments which reach from one blastomere to another, and remain for any length of time, there would be a greater or less transference of living material. Segmentation in such eggs cannot be regarded as a separation of the egg substance into independent "cells," but becomes rather a differentiation of different portions of the same unit mass for different purposes.

2. If spin-threads are outflows of internal protoplasm, their activities must be similar to those in the interior of the egg—a conclusion reached from other material by G. F. Andrews (4).

One of the activities of the spin-threads is a streaming movement of their more liquid contents. Unfortunately the blastomeres are so opaque that we cannot see whether there is a corresponding internal flowing of the protoplasm, but it hardly seems possible that the former could exist without the latter. Such streamings of the egg protoplasm have been witnessed by Erlanger (21) in the living eggs of several small Nematodes which are transparent enough to show it distinctly.

In each end of these eggs alternately the protoplasm moved from the poles to the equator, but as soon as the equatorial groove appeared the streaming turned inward at the groove and then backward toward the pole. After the first segmentation similar streamings appeared in the blastomeres. These movements were vigorous enough to distort the cleavage spindle, and Erlanger concluded that the whole of the egg, spindle and astral rays included, is always plastic and fluid, though the rays are more viscid than the remainder. Blunt pseudopodia were formed on these nematode eggs at various points, especially near the edge of the cleavage groove, but no spin-threads were noticed.

The observation of such phenomena in transparent eggs furnishes a strong presumption in favour of their occurrence in opaque eggs, particularly in connection with such vigorous external movements.

3. The spin-threads and other cytoplasmic connections assist materially in appression, and probably in other movements of the blastomeres.

The spin-threads possess a contractility of sufficient strength to move the blastomeres, and there can be no doubt that they assist in drawing them together after segmentation.

They produce visible motion after the first cleavage, but not in any succeeding stage, because the physical conditions

are not the same. But it is not unreasonable to suppose that the filaments formed in such stages really do assist in appression, even if we cannot see any motion. A similar function has been pointed out for the connecting filaments formed in Echinoderm eggs (**5**). It seems probable also that something of this sort produces the spiral movement during the third segmentation. We must admit that some portion of the internal protoplasm possesses contractility, or we are again driven to the improbability that the protoplasm which streams outward to form the spin-threads is radically different from that which remains inside the egg, and that the change takes place at the ectosarc. We thus see that all the facts and inferences to be derived from the visible filose activities give support on the one hand to the hypothesis of fibrillar contractility (van Beneden, **9**), and on the other to the explanation by means of streaming or osmotic movements of the fluid portions of the protoplasm (Bütschli, **12**). Neither of these views can well be excluded in face of the testimony here presented.

It seems equally impossible to escape the conclusion that both are correct, so far as the existence of the forces which they advocate is concerned. May it not be that we are to look for a full explanation not to either one of them alone, nor to any other single hypothesis already advanced, but rather to a compromise, including at least several of the principal ones?

Gastrulation.

Ciliation of the Larva.—As soon as the blastula stage is reached cilia appear upon the external surface of the cells, and the embryo begins to rotate slowly about a vertical axis.

The displays of filose spinning already given by the polar bodies and the blastomeres incline me to the view recently expressed (**4**) that cilia owe their origin to similar activities.

During segmentation both polar bodies and blastomeres were sending out spin-threads, which often traversed spaces

of considerable width. What more natural than that this same activity should manifest itself at the close of segmentation in spinning out these cilia? Evidently they cannot be extrinsic in their origin, but must be intrinsic. Various names have been applied to them, but I would suggest that the term filature expresses better the actual facts in the case—i. e. a spinning out into threads of filose processes.

These cilia vary greatly in length, the majority of them being far longer than those ordinarily given in published drawings (see **4**, p. 72). Furthermore they do not end abruptly at a definite distance from the cell surface, but terminate in fine processes similar to the terminations of the spin-threads from the polar bodies. I have ventured to represent these cilia in one drawing as they really appear (fig. 8), and I believe that such figures reveal their true nature.

Such a relation between spin-threads and cilia may be taken in evidence to explain the origin of motion in the latter.

The band of strong cilia around the edge of the oral surface are probably the ones first developed. At the close of segmentation there is a well-defined differentiation of cells into ectoderm and entoderm. The former are considerably the smaller, and are more or less cubical in shape, while the latter are elongated into well-defined cylinders (fig. 69).

During the blastula stage the egg maintains a position in which the entoderm cells occupy the inferior pole, while the ectoderm cells occupy the superior hemisphere and the upper part of the inferior one. The lower surface becomes somewhat flattened previous to gastrulation, and it is in the ectoderm cells bordering this flattened area that cilia are first seen (fig. 5).

The difficulty lies in proving that these cells maintain their original position through gastrulation.

Formation of the Gastrula.—Toward the close of the twentieth hour the large entoderm cells at the inferior pole turn inward and upward. The flattened area around this

invagination becomes the oral surface, while that which is for the present the superior portion of the embryo is the aboral surface. These correspond to the terms "sub-umbrella" and "umbrella," as used by Salensky (**45**). The opening in the centre of the oral surface—the blastopore—is the only opening into the invaginated entoderm. It is permanent, and functions as both mouth and anus during the life of the larva, and is probably transformed into the mouth of the adult as in other species, a new anus being formed. The entire inner surface of the entoderm is covered with cilia as soon as they appear on the ectoderm. The cilia around the edge of the oral surface are much larger and longer than elsewhere on the pilidium, and form what is known as the pre-oral ring, which corresponds with those on trochophore and turbellarian larvæ.

At this period the larva consists of a single outer layer of cubical ectoderm and an inner layer of invaginated columnar entoderm, also single. The space between these—the body-cavity—is filled with a colourless, transparent, gelatinous liquid.

Invagination is thus embolic, and the first stage in the formation of the pilidium is accomplished.

The Apical Plate.—As soon as invagination becomes distinct the embryo falls over on its side. At the same time a slight depression appears near the centre of the aboral surface (fig. 8), which is saucer-shaped at first, but becomes markedly cup-shaped in later development. It never lies exactly at the centre, but always a little anterior to it on the median line.

For this reason it marks the first appearance of bilateral symmetry in the embryo, which has hitherto preserved a distinctly radial form (cf. Korscheldt and Heider).

In the centre of the depression there is developed what at first sight appears to be, and what was long mistaken for, a single very strong flagellum. It is really a bunch or tuft of flagella, twenty to thirty in number, which are nearly always held firmly together. The depression, the apical plate, is

made up of ectoderm cells much smaller than the average, and elongated at right angles to the surface. In its formation the cubical epithelium becomes columnar and invaginates into the body-cavity as a thimble-shaped papilla. As invagination proceeds the columnar cells become wedge-shaped, with their bases toward the body-cavity, and, withdrawing a little, leave a depression at the centre of the papilla, which furnishes the requisite support for the flagella. The latter arise singly from the inner ends of the wedge-shaped cells, and are wholly unlike the rowing flagella possessed by so many larvæ, never being used for locomotion. They are rather enlarged cilia, soft and very flexible, and they originate, so far as can be determined, like the cilia, by a spinning out of the protoplasm from within the ectoderm cells. Consequently they are sensitive, and serve as tactile organs of considerable delicacy and power.

The Change from Radial to Bilateral Symmetry.—It has just been stated that the apical plate is eccentric in position.

This first appearance of bilateral symmetry is quickly emphasised by an elongation of the embryo parallel with the eccentricity, and the long diameter thus determined becomes the longitudinal axis of both larva and adult. In later development the entodermal invagination is elongated along this same axis, but in one direction only, i. e. away from the apical plate.

This completes the differentiation, and we are able to distinguish anterior and posterior, dorsal and ventral, right and left, and the second stage in the formation of a pilidium is complete.

Such a transition from radial to bilateral symmetry is of interest in its bearing on phylogeny. Embryologists generally agree that several of the largest groups of Metazoa show enough resemblance in their larval development to warrant the conclusion that they are all descended from a common ancestral form. These groups include Annelids, Molluscs, Rotifers, Gephyreans, Nemerteans, and possibly others.

There have been several attempts to arrive at an idea of their ancestral form, but the way is beset by so many difficulties that the attempts agree in few particulars. These few, however, are significant, for it is conceded that the archetype must have been very simple, that it possessed radial symmetry, and probably approached closely to a uniformly ciliated gastrula, with a rounded aboral surface and a flattened oral surface, in the centre of which was situated the mouth. Around the edge of the oral surface was a ciliated ring, possibly innervated.

Even in these meagre details we recognise almost an exact description of our Cerebratulus larva, which is, like that of many Nemerteans, developing through a pilidium.

The only noteworthy difference is the presence in the pilidium of the apical plate with its bunch of flagella, which forms a well-developed sense-organ. But in several important respects the pilidium larva is less highly developed than any of the others mentioned. This is especially seen in the form of the alimentary canal, in the absence of an anus, and in the lack of any true nervous system. In these particulars it approaches the ancestral type more closely than any existing larva, and in consequence the manner in which it changes from radial to bilateral symmetry becomes of interest.

The flattening of the inferior pole and the establishment of the gastrula invagination leads to the fixing of a structural axis passing vertically through the mouth, with the whole body arranged radially around it. But the next step is not an elongation of the aboral surface and the formation of an anus there, as assumed for the ancestral metamorphosis.

It is rather an unequal elongation of the oral surface, whereby the portion in front of the mouth forms the pre-oral lobe, while that behind the mouth develops into the trunk and forms an anus.

Thus the aboral surface retains its relative position and becomes the dorsal surface of the Nemertean.

The Escape from the Egg Membranes.—Cilia have now developed over the entire external surface of the larva, and as soon as it falls upon its side the rotary motion which they produce presses the aboral surface against the membranes.

This elongates the latter into an ellipsoidal form, and bends the tuft of flagella at a sharp angle near their base.

By continued rotation this sharp angle is driven against the membranes forcibly enough to bend them outward in a shallow dent (fig. 6). This keeps the sharp angle in position, and it soon bores its way through the membranes (fig. 7).

The bunch of flagella is then straightened out, tearing the edges of the rent still more, and some of the liquid which filled the membranes escapes. This causes them to collapse, and they fall against the rotating embryo, whose strong motion quickly tears them in pieces, leaving the larva free.

This is the second and probably the most critical period in the life of the embryo. Of those reared from artificially fertilised eggs fully one third fail to escape from the membranes, and normal embryos fare no better, since 35 per cent. of those under observation perished inside the membranes. When we reflect on the apparent ease with which the sperms penetrated these same membranes we are impressed with the delicacy of the apical flagella. Hatschek has noted in the Annelid *Eupomatus cruciatus* that the cilia pierce the membrane when they first appear, and it would seem as if the latter was not as tough as in Cerebratulus (Korscheldt and Heider, p. 173).

We find examples of both dextral and sinistral rotation, with a large majority in favour of the former.

The rapidity of rotation and the time at which the larva escapes also vary greatly. If unable to escape at the proper time the continued rotation reacts upon the larva and tears asunder the cells of which it is composed.

In consequence a ragged bunch of cells may often be seen rotating in the midst of a loose mass of individual cells which have been separated from it, the whole enclosed in the original membranes. Or sometimes the membranes give

way at the last moment, and the bunch of cells is set free rotating slowly.

The Pilidium.

Habits.

Locomotion.—The larva always moves with the aboral surface ahead, as seen in fig. 8. Locomotion is accomplished wholly by the cilia, particularly the band of large cilia around the edge of the oral surface. Their combined action results in a uniform and quite rapid forward motion, and there is also a continuance of the old rotary motion, though the axis of rotation is now horizontal instead of vertical.

The life of the larva seems to depend on its constant motion, and if one is stopped for any length of time it goes to pieces, the cells being torn asunder by a vigorous contraction of the body muscles. This renders any extended examination of living specimens very difficult. Nevertheless it was determined to examine these larvæ alive for the reason that they become beautifully transparent immediately after gastrulation, so that internal development can be followed in detail.

After repeated trials it was found that the best method of keeping the larvæ quiet was to place them in a drop of salt water under a supported cover-glass, and then paralyse them with cocaine, or better with chloral hydrate. If just the right amount (which must be very small) be used the larva will remain stationary for a long time, with the cilia moving slowly and without any contraction of the muscles. In this condition camera lucida drawings are easily made, and the morphology which follows has been obtained entirely in this way from a study of living larvæ.

Use of Apical Flagella.—In swimming the long flagella are stretched far out ahead, and move round slowly in front of the advancing embryo. So completely are these flagella controlled by the apical muscle that they are capable

of movements in every possible direction and of great delicacy.

The larva is often seen advancing toward some object, or toward the outer surface of the drop of water in which it is swimming, and when still distant from it half its own diameter it turns suddenly, sometimes even completely around, and escapes coming in contact with it. Either, therefore, the flagella are sensitive enough to recognise an object when still some distance from it, or they are prolonged beyond their visible tips, the prolongations being invisible under the power used.

In view of what we have already seen we must conclude that the latter is far the more probable.

The flagella also serve as a steering apparatus, like the tuft of cilia at the apical pole of many Actinian larvæ, and the apical plate in its earlier origin is sometimes traced back to such a rudder-like tuft of cilia (cf. Korscheldt and Heider).

Here in the pilidium we find both apical plate and apical tuft, the latter retaining its original function of steering.

Farther than this the flagella take no part in locomotor movement, but are pre-eminently tactile, and constitute practically the only sense-organ possessed by the larva.

Their importance is shown by the comparatively complex structure of the apical plate from which they arise, and by the attachment to it of the largest and best developed muscle in the whole pilidium. And yet they are exclusively a larval organ, and take no part in the formation of the adult worm, being thrown off with the epithelial covering.

Sometimes there are two apical tufts instead of one as in the figure here given. In such a case the plate is broadened and thickened, but there is only a single apical muscle, developed in the usual way.

Pilidiums are sensitive to light, always congregating on the side of the aquarium nearest the source of light, but they show no response to ordinary sounds.

Food.—The pilidium swims at the surface of the water

until its transformation into the adult form. During this time its food supply is varied. It consists chiefly of very small particles of the refuse material always found in sea water; in no instance were living organisms found in the digestive organs. The cilia on the inner surface of the entoderm keep up a constant current inward through the single opening at the centre of the oral surface, and another intermittent current outward. The former runs along the anterior side of the invagination and carries with it the food particles. In the stomach the current becomes more or less rotary, carrying the food round rapidly until all that is digestible has been absorbed. The refuse material is caught from time to time in the intermittent current and carried outward along the posterior side of the invagination, and discharged by the same opening at which it entered. This single opening, the blastopore, thus functions as both mouth and anus. Often the refuse is thrown to quite a distance by a vigorous flap of the lappets, the larva at the same time being driven forward with a jerk. After the primitive intestine becomes differentiated into stomach and œsophagus the opening between the two is strongly constricted by a sphincter muscle, which operates exactly like the pyloric sphincter in our own bodies.

Morphology of the Pilidium.—As the larva elongates in passing from radial to bilateral symmetry two enlargements appear, one on either side of the blastopore, growing downward from the border of the oral surface. These form ear-like lappets, diverging slightly from each other, and the larva has now assumed the helmet form of a typical pilidium. This species is slightly elongated antero-posteriorly, and the lappets are quite thick (figs. 9, 10).

The whole pilidium is built up of the three primitive tissues nearly unmodified. The ectoderm forms the skin, the entoderm the intestine walls, while the mesoderm fills the space between. The gradual development and transformation of these three tissues takes place very slowly, and affords a good opportunity for studying the tissues themselves and

their derivatives. The entire surface of the pilidium is covered with cilia, those along the edge of the helmet being thicker and stouter than elsewhere, since the pre-oral ring of the gastrula is continued around the edge of the lappets. By a contraction of the muscles with which the lappets are soon supplied a peculiar flapping movement is produced, very similar to that of a pecten's shell when swimming, and this seems to be of assistance in locomotion. The lappets are also quite sensitive, though they are always carried behind the larva when swimming, and hence cannot be of as much service as the flagella.

The larva has now become perfectly transparent, so that the details of internal development can be easily watched.

The Primitive Intestine.—At first no differentiation is possible in the invagination which forms the primitive intestine. It is composed throughout of a single layer of cells which are longer and narrower than those of the ectoderm, and which bear cilia upon their internal faces. These cilia are very delicate, and are manifestly the result of filose action. As soon as the embryo elongates, however, the invagination bends over toward one end, the future posterior end of the embryo (fig. 10). This flexed end approaches close to the wall of the pilidium, enlarges, and assumes a spherical shape.

A slight constriction appears at the point of flexure, and we can now distinguish an œsophageal portion which extends from the mouth to the point of constriction, and a stomach portion, the enlarged inner end. Histological examination reveals a clear distinction in structure also between the two (cf. p. 188).

As the stomach enlarges the aboral surface posterior to the lappets becomes larger than the corresponding anterior portion.

Consequently it rounds over somewhat where it joins the oral surface, and the latter sinks in as a shallow saucer-shaped depression, approaching closely to the wall of the stomach. But the two never meet as in the trochophore larva,

At first the œsophagus is cylindrical and of the same diameter throughout, but later it is enlarged laterally and constricted around the mouth as well as at its junction with the stomach. This dilation produces a lateral elongation of the mouth opening, which becomes more and more elliptical.

Both œsophagus and stomach walls are covered with muscle-cells. In the former muscles are formed, which, by inflation and constriction, assist in taking in and ejecting the food, and probably also in breathing. For in view of the fact that the adult breathes through these same œsophagus walls, and that a strong current of water is kept flowing over them by the action of their cilia, it seems rational that oxygenation should take place here in the larva. On the stomach the muscles do not seem strong enough to render much assistance in churning the food, but at the constrictions they develop into strong sphincter muscles. The one around the mouth in other species becomes the lip muscle of the adult worm.

The simplicity of this primitive intestine is the more remarkable when we reflect that it is the only portion of the pilidium which is preserved in the adult Nemertean.

Mesoderm Formations.—These include mesenchyme cells and the whole musculature of the larva. In no other larva with which I am acquainted can the development of the muscular system be followed so easily and in such detail.

This is due to a combination of peculiarly favourable circumstances. First the pilidium walls are as clear as glass, enabling the single mesenchyme cells to be seen through them from the very beginning. Then the mesenchyme cells themselves are large, and as soon as they become polar can be readily distinguished from all others. Finally the development into muscle is so gradual, and the ultimate tissue is so simple, that a single cell may be followed for some time after it has united with others, and has begun to function as muscle.

Consequently the development of the muscle system of the pilidium larva is one which discloses something of the real

nature of muscle tissue as to its origin and functions. And yet, so far as known, the present is the first attempt to work out this development in any pilidium. Even Bürger (**13**), in his excellent monograph, can only say, "The mesoderm gives rise to the gelatine, the star-shaped cells lying in it, and the muscle fibrillæ. The latter have doubtless developed out of the star-shaped cells."

Origin of the Mesenchyme Cells.—Metschnikoff has shown in the genus Lineus (**35**) that the mesenchyme originates from large entoderm cells around the border of the invagination, and the same is true in Cerebratulus. When gastrulation first begins, scattered mesenchyme cells separate from the entoderm in the angle between the invagination and the oral surface, and sometimes along the convex invagination itself (fig. 72).

Unlike Lineus, there are here two kinds of these cells, readily distinguished by their size (fig. 73).

The larger ones are given off first, and for a little while are the only ones to be seen; then both kinds come off together indiscriminately. We will call them macromesencytes and micromesencytes respectively.

At first the macromesencytes are rounded in form, and float about freely. At this time they are difficult to discern against the background of ectoderm, but they soon become branched, and are then easily distinguished from all other tissue.

The branches hinder their freedom of motion enough to show that the liquid in which they float is gelatinous rather than watery, and they gradually become fixed in position and function as muscles. While free they exhibit amœboid movements, similar to those of blood leucocytes; then the cell puts out slender, blunt pseudopodia, which change their position, size, and shape, like those of an amœba. But they quickly become fixed in position, and are then spun out much farther, with tips so attenuate that it is impossible to tell where they really do end.

They continue to increase in length until they finally touch

the body-wall, or that of the intestine, to which they fasten themselves. They then function as muscle by contracting and pulling together the parts to which they are attached.

While they remain free no trace of striation can be seen in the cells, but we have to remember that we are looking at them through the pilidium wall, which, however clear it may be, is not perfectly diaphanous. Hence there might exist a fine striation without our being able to detect it. But as soon as the cells become fixed in position granules appear in the cell cytoplasm, and the thread-like branches become distinctly fibrillar in structure. No amitotic division stages were found, as noted by Montgomery in the free mesenchyme cells of the adult worm (**37**). Occasionally the branches of two cells anastomose before becoming attached, and may be seen to contract, at first irregularly, but later rhythmically, like similar cells in opisthobranch Gastropods (cf. **49**). After the cell becomes fastened to the pilidium walls these rhythmic pulsations cease, and the cells become regular muscles, contracting only when stimulated. The muscles developed from these macromesencytes are considered under the following heads:

1. Retractor Muscle of the Apical Plate.—This is usually the first muscle formed. Soon after the intestine has turned down posteriorly mesenchyme cells are seen along the apex of the bend beneath the apical plate (figs. 8, 9). One or more of them become stationary at about the position of the future muscle.

It then sends out spin-threads which reach, and fasten themselves to, the apical plate, the wall of the intestine, and sometimes the aboral wall of the pilidium. Other cells in floating about come in contact with these fixed ones, and are arrested. They in turn send out processes to the apical plate, the intestine, or the wall of the pilidium, according to their position. Other processes are then sent out which anastomose with those already formed, making a coarse network (fig. 11).

The strands of the network are then pulled around by the

formation and attachment of new processes and the letting go of old ones, until they are approximately parallel (fig. 14). The strands then come together and fuse into larger ones, while the cell bodies, which now contain little beside the nuclei, travel slowly along the strands, and seem finally to lie on the under surface of the apical plate, or upon the wall of the intestine.[1]

The removal of the nuclei leaves the strands in the condition of clear muscle-fibres (fig. 15). Occasionally the body of a cell is all used up in forming processes, and then the nucleus may be flattened slightly and retain its place in the fibre.

The muscle is thus fastened first to the wall of the intestine (fig. 13), and is so figured by Verrill (**46**, p. 417).

But it soon divides and passes down on either side of the intestine, and fastens to the anterior border of the lappets, separating into fine fibres at the point of junction (fig. 15).

In a good light the fibres may be seen to be attached to delicate papillæ, which are raised slightly from the inner surface of the ectoderm cells. The muscle is now no longer connected with the stomach, for a strong contraction draws the apical plate down until it almost, if not quite, touches the stomach wall. This is the longest, the largest, and the strongest muscle in the pilidium, as well as the first one to be formed, and it is chiefly through its violent contractions that the cells of the pilidium wall are pulled asunder under irritation.

In such a case, after the muscle has shortened and thickened all its structure will allow, a still further shortening is produced by the assumption of a corkscrew shape, exactly as in the contractile stem of Vorticella. This is the only means by which the muscle can draw the apical plate down far enough to touch the stomach wall.

2. Interparietal Muscles.—The first of these consists of a single cell, which lodges usually just in front of the apical plate (fig. 17). Long processes are developed at

[1] Mrs. G. F. Andrews kindly gave me this final location of the nuclei, from her own observations upon Cerebratulus.

either end of this cell, which extend across and fasten to the pilidium wall.

At first the fibres are simple, and contraction results in pulling the pilidium walls closer together; but in later development they may be joined by other fibres, and the motion then becomes compound, producing a general shrinking of the body-walls.

There are also one or more cells which sometimes accompany the large retractor muscle, but whose fibres only extend from the apical plate to the wall of the intestine (fig. 15).

These are insignificant in size and strength, and probably play an equally insignificant part in the motions of the larva.

Finally, there are muscles, consisting mostly of single cells, which run from different points of the pilidium wall to that of the intestine (figs. 13, 15). These seem to develop wherever the cell is finally lodged, and their contraction produces movement in both the walls to which they are attached.

Being formed from a single cell, the body of the cell usually remains visible for some time, but later it may travel along to one wall or the other, and leave a simple strand stretched across the intervening space. Here, then, we witness the transformation of a cell, at first independent of all the rest of the larva save the liquid in which it floats, into a muscle-fibre fixed at both ends, and capable of contracting under stimulus.

3. Œsophageal Muscles.—There are two sets of these:

a. Gastro-œsophageal, consisting of delicate fibres running diagonally between the adjacent walls of œsophagus and stomach.

Most of them consist of a single macromesencyte and its branches; they do not anastomose, and are so far apart that they cannot fuse (fig. 14). They are not formed until the seventh or eighth day, and are used chiefly in the dilation (antero-posterior) of the œsophagus. They are weak, and scarcely worth a separate classification.

b. Post-œsophageal, a thin muscular sheet, triangular in shape, connecting the oral ectoderm with the posterior

wall of the œsophagus along its mid-line. Sometimes the sheet is divided, and attached along either side instead of in the mid-line.

In fig. 14 several mesenchyme cells are seen just fastened in place and putting out processes; in later development (fig. 15) the individual cells have coalesced into a broad triangular sheet of muscle. One side is attached to the œsophagus from the mouth nearly to the stomach; a second side is attached to the ectoderm from the mouth back to the angle between the lappet and the oral surface; while the third side, somewhat concave, hangs free. The bodies and the nuclei of the individual cells composing this muscle can be seen for a long time as swellings irregularly distributed through the muscle sheath.

The œsophagus is ordinarily dilated laterally, so that the contraction of this muscle dilates it antero-posteriorly, and, when followed by a contraction of the muscular walls of the œsophagus itself, aids materially in generating the currents of water which take in the food particles and eject the waste matter.

The motion thus produced corresponds also to the breathing movements in the adult worm, and it does not seem rash to suppose that the larva also breathes in this way, and that the muscle under discussion is one of the muscles of respiration.

4. Circumoral Muscle.—This consists of a strong muscular ring which surrounds the mouth, and of muscle bands which extend backward and forward from the ring to the edge of the lappets (fig. 15). The elliptical mouth extends from the centre of one lappet to that of the other, and across the space intervening between the lip and the anterior and posterior angles of the lappets numerous muscle-fibres are developed. At the side are usually found large cells, which send out fibres reaching to the extreme anterior and posterior walls of the pilidium, as well as shorter lateral ones. These cells are well developed, and their contraction tends to draw the borders of the oral surface in toward the centre. The ring around the mouth is developed into a well-defined

sphincter, whose contraction can almost close the mouth opening. These ring and band muscles form an excellent support for the attachment of the other muscles, notably those of the lappets and apical plate, and for the support of the primitive intestine. In fact, they serve as a sort of centre for the whole body musculature; and this seems an important use under normal conditions. They also restore the œsophagus to its normal shape after dilation, and, next to the apical muscle, are the most strongly constricted under irritation. They have the advantage of being situated close to the source of supply of mesenchyme cells, and in other species they are retained during metamorphosis, and develop into the strong lip muscles of the adult Nemertean.

The second kind of mesenchyme given off by the entoderm consists of much smaller cells than the first. Very soon after the appearance of the large cells these smaller ones may be seen coming off in the same locality. The difference in size is shown in fig. 9, and is not diminished by subsequent growth. These micromesencytes do not float about like their larger predecessors, but remain close to the body-wall (fig. 14).

They do move about, however, in a curious way.[1]

After being warped about in different directions they each settle down at some particular spot, and become permanently attached, to form the fifth group of muscles. Conn, in his study of Thalassema (17), found two kinds of mesenchyme cells, one of which became distinct muscles, while the other consisted of branching cells of the same origin, but more like "connective tissue corpuscles scattered at random in the body-cavity, but quite close to the body-wall. In the older larvæ they are more abundant, and unite to form quite a continuous layer." Their similarity in origin, form, and position to the micromesencytes of Cerebratulus, and the fact that they are ultimately joined in a continuous layer, suggest that they may have a similar function.

[1] In a verbal communication Mrs. G. F. Andrews states that she has seen these cells warping themselves about by means of protoplasmic processes.

5. Parietal Muscles.—These consist of single micromesencytes scattered over the entire inner surface of both ectoderm and entoderm (fig. 16). Their fibres anastomose freely with one another and with the interparietal muscle-fibres, and form an irregular network, which is attached closely to the inner surface of the pilidium walls. Their contraction may assist the motion produced by other muscles, or may produce a local shrinkage something after the manner of the so-called "goose-flesh" on our own skin under the influence of cold.

Certain of the cells on the walls of the œsophagus are arranged with more or less regularity transversely, and form constrictor muscles. It is possible that some of the macromesencytes assist in the formation of these constrictors, since some of them are attached in the right position and afterward disappear, but the matter could not be definitely determined.

These muscles are usually incorporated in the tissue of the intestine wall during subsequent development, and together with many of the macromesencytes which have remained isolated are transferred to the adult Nemertean during metamorphosis, as has been shown in other species by Bütschli (**11**). All the remainder of this quite intricate musculature disappears.

The parietal network forms a layer separating the ectoderm from the central gelatine, and assists the other muscles in pulling asunder the cells of the pilidium walls under irritation.

We thus see in the musculature of the larva which is temporary, and the bulk of which disappears during metamorphosis, a curious foreshadowing of the function of dismemberment which is so prominent in the adult.

6. Lappet Muscles.—These muscles necessarily await the development of the lappets, and are thus the latest to appear, coming about the eighth day. The cells from which they originate are almost of the same size, and become stationary close to the cilia ring, forming a row just inside the cilia cells (fig. 81).

Each cell then sends out a process which develops into a muscle-fibre. There are two sets of these lappet muscles.

a. Radial Muscles of the Lappets.—These consist of fine fibres extending radially from the edge of the lappets to the circumoral ring (fig. 15). They are all of the same size, and are arranged at approximately equal distances from one another, each micromesencyte forming but a single fibre.

They anastomose little, if at all, but each fibre at the edge of the lappets divides into from two to five fibrils.

Each fibril is attached to a fine mammillary process on the inner surface of a cilia cell, which looks like a miniature of one of the papillary muscles of the chordæ tendineæ of the human heart. These muscles correspond to the "large muscles of the lappets" noted by Salensky in Lineus (**45**), but differ from them in that they do not radiate from a single point.

There is a small group (fig. 15) radiating from the same point as in Salensky's figure, but it does not constitute an important part of the muscle. The actual process of attachment by which one of the muscle fibres is fastened to the pilidium wall is difficult to watch, but it is easy to determine in these lappet cells that the muscle fibrils are connected with the cytoplasmic reticulum. When one of the threads sent out by a mesenchyme cell comes in contact with an ectoderm cell it first flattens out a little on the surface of that cell.

The substance of the thread, which is actively moving protoplasm, then fuses with the ectosarc of the cell, and through it becomes attached to the reticulum in the interior. That it is certainly thus attached is seen upon the disruption of the pilidium under irritation, when muscle fibrils may sometimes be found with a single ectoderm cell still attached to either end.

These of course were interparietal muscles. If now the cells be held in position by pressure of the cover-glass the muscle-fibre will go on contracting for some time.

By increasing the power used until the reticulum of the

cell becomes visible, it can be seen that the threads of this reticulum are apparently continuous with the fibrils, and that every contraction of the latter pulls the reticulum adjacent to the point of attachment out of place. As soon as the contraction ceases the reticulum returns to its former shape. The ground substance of the cytoplasm is apparently affected only as it is compelled to accommodate itself to the changes of shape in the reticulum. The cytoplasm of the thread, therefore, at first fuses imperceptibly with that of the cell to which it is being attached, but on becoming fibrillated the fibrils attach themselves to the more substantial reticulum.

These radiating fibres are very contractile, and may often be seen to assume the same corkscrew shape as the apical muscle when excessively contracted. They serve to produce the opening and closing motion of the lappets already described.

b. Locomotor Muscles of the Cilia Rows.—These correspond closely to the circumoral muscles in origin and mode of development. The cilia rows lie at the junction of the aboral and oral ectoderm where the mesenchyme cells originate.

When the lappets first start some of these cells are taken along with them in their downward growth, and retain their original position just inside the cilia cells (fig. 11). From a portion of these micromesencytes are developed the radial muscles just described, but most of the fibres unite to form a muscle strand running along inside the cilia cells. This strand increases as the lappets enlarge, and unites at either end with the circumoral muscle (fig. 77). Its fibres divide into fibrils which terminate in the individual cells of the cilia rows.

The strand can be plainly seen in all longitudinal or diagonal sections which cut through the edge of the lappets, and it may be detected in specially perfect cross-sections, where of course the fibres appear as dots. These are the only muscles of the larva distinctively for locomotion (cf. p. 159).

But not all the mesenchyme is employed in the manufacture of muscle. Certain of the macromesencytes do not develop processes or exhibit amœboid movements, but divide and subdivide actively. As fast as they are used up in this way others take their place, so that they may always be found scattered about in the gelatine. In the living larva they appear yellowish, and they always stain more deeply than the muscle cells. Evidently they are the source of the gelatine itself, and probably correspond to the isolated cells which Bütschli showed (**11**) were transferred to the adult Nemertean. If their development could be followed in this species it would doubtless be found that they share the same fate.

Summary.—1. The mesenchyme originates from large entoderm cells situated at the junction of entoderm and ectoderm.

2. Its cells are of two kinds, called macromesencytes and micromesencytes respectively, the former being several times larger than the latter. At first macromesencytes are given off alone, then both kinds appear together. From micromesencytes are developed the parietal and lappet muscles; from macromesencytes all the other muscles of the pilidium.

3. In this muscle development both kinds of cells may either remain single, forming delicate fibres, or may anastomose into a complex network, or several cells may fuse into a larger strand.

4. At first the macromesencytes float about freely and exhibit slight amœboid changes of form. Then they put out slender pseudopodia branches which arrest their motion. These pseudopodia do not show any contractile movements, but only a streaming motion of granules and cytoplasm. They are lengthened until they come in contact with something to which they can be fastened.

5. As soon as they are fastened the structure changes from that of normal cytoplasm into typical fibrillated muscle. This is accompanied by a corresponding change in activity; the streaming ceases, and the fibrils become contractile and function as muscles.

6. Prior to fixation two macromesencytes sometimes anastomose with the same change in structure and activity, but they pulsate more or less rhythmically instead of contracting in the ordinary manner. As soon as they fasten to the larval walls, however, the pulsations cease, and their subsequent contractions are like those of other muscles.

7. The cytoplasm of the macromesencytes is exhausted in the formation of branches. The nucleus retains its original position until the muscle-fibres are formed; it may then become flattened and incorporated in situ in the muscle tissue, but more frequently it migrates along the fibre to the pilidium wall.

8. The micromesencytes do not float about, but remain close to the pilidium walls, upon which they creep along to their final position by means of "protoplastic activities."

9. The ends of the thread-like pseudopodia sent out by both kinds of mesenchyme, on coming in contact with a cell of the pilidium wall, at first fuse with its ectosarc, but after they become fibrillated the fibrillæ are attached to and are apparently continuous with the cell reticulum.

10. The ectoderm and the entire musculature of the larva, except the parietal muscles of the intestine and a few isolated macromesencytes, disappear during metamorphosis.

11. When irritated the larval muscles contract violently and pull in pieces the walls of the pilidium, thus showing the same tendency toward dismemberment that is manifested in the adult.

Conclusions.—The simple fact that circumstances combine so favourably for a study of the musculature in the pilidium would give it considerable interest. And when we remember how closely it approaches in primitive simplicity to the archetype of several of the largest groups of Invertebrates, it becomes doubly interesting, and we are enabled to draw several suggestive conclusions.

1. The muscles of the pilidium are metamorphosed pseudopodia.

The pseudopodia of the mesenchyme cells are the exact

counterpart of the spin-threads sent out from the polar bodies and from the blastomeres during segmentation. Some at least of those spin-threads were contractile, while these mesenchyme spin-threads actually develop into typical muscles. The same thread which at first exhibits a visible streaming of its cytoplasm afterwards becomes contractile and shortens just as visibly.

2. The transition from pseudopodium to muscle-fibre consists essentially in a striation or fibrillation of a portion of the cytoplasm, and the gradual assimilation of the non-fibrillated remainder.

Such a striation of cytoplasm is nothing new. It has been observed by Flemming and many others in the living cells of cartilage, epithelium, connective-tissue and other animal cells, in which the fibrillæ form a sort of loose network. It has been seen by G. F. Andrews in living cells of many Protozoa and Metazoa (**4**, **5**), where it was plainly correlated with contractile activities. In preserved material striation is shown in the pancreas cells of Necturus (**51**) and in the beautiful preparations of ciliated cells from Anodonta and Cyclas (**20**).

But the very best examples are those given by Heidenhain (**24**) of the radiating system of fibres in the leucocytes or wandering cells of the salamander, and by Zimmermann (**52**) of similar radiating systems in the pigment cells from the epidermis of fishes. Here the cytoplasm not only becomes fibrillar, but both authors believe it to represent the contractile elements by means of which the cells change their form and creep about (**51**).

In all these cases the cytoplasm becomes fibrillar inside the cell, but that ought not to exclude the possibility of its repeating this tendency outside the cell in the pseudopodia.

Conn states (**17**) that in the mesoderm formations of Thalassema the cells which are budded off from the entoderm near the blastopore do not become true wandering cells, but are immediately transformed into muscles. Each muscle is

unicellular, consisting of a cell body with a distinct nucleus, and with muscle-fibres extending from it in two directions. Here, then, the branches become fibrous as well as the body of the cell.

When the streaming movement necessary to the sending out of spin-threads is stopped by contact with something, the threads at once become striated. At first the striæ are hardly visible and some distance apart, but they rapidly increase in size and distinctness until finally they occupy nearly all the muscle strand. It seems probable that this increase in fibrillation consists largely of an assimilation of the non-fibrillated portion. During this transformation the whole cell body is often conveyed out into the incipient muscle strand, where it is converted into muscle fibrillæ.

3. Fibrillation appears to start in a rearrangement of the cytoplasmic reticulum, whereby its threads, instead of forming an irregular network, become parallel.

We can see how a streaming of the cytoplasm along the spin-thread pseudopodium would help such an arrangement, for the fibrillæ are longitudinal. Several other facts support such an origin of muscle fibrillation. The familiar radiating fibrillæ in the asters of dividing cells, when followed outward, can be traced into the cytoplasmic reticulum, and they are so continuous with its threads that it is impossible to tell where the fibril ends and the reticulum begins, or vice versâ.

Again, the muscle fibrillæ under favourable conditions can be followed into the cell to which they are attached, and terminate there in the cytoplasmic network, with which they are so completely fused, that once more it is impossible to tell where one ends and the other begins. Furthermore, in a study of columnar epithelium it may often be noticed that the cytoplasm in the outer zone of the cell has been arranged in more or less parallel fibrillæ along the long diameter of the cell. No reticulum is visible in this part of the cell except that formed by the fibrillæ. In figures of

ciliated epithelium given by Englemann (20) the fibrillæ are almost perfectly parallel, and not only correspond exactly in number with the cilia, but are just opposite to them. We can hardly escape the conclusion that the external cilia are prolongations of the internal fibrillæ. If so, then the difference between cilium and muscle-fibre is that the former consists of a single fibrilla, while the latter is made up of several bound together.

Finally the fibrillæ, when they first appear, are not upon the surface of either cell or spin-thread, but are distributed throughout their substance. Hence they cannot be regarded in any sense as "surface secretions," but are more intimately associated with the cytoplasmic structure. If not formed by a rearrangement of the reticular network, they at least bear the same relation to the remainder of the cytoplasm that such threads would.

4. The first contractions of the changing pseudopodia represent the combined action of all cytoplasmic energy, pulling in a single definite direction instead of in different directions.

Originally the cytoplasm possesses perfect internal mobility, and its first movements are amœboid in character. Then follow spinnings, or the putting out and withdrawal of pseudopodia in different directions. Some of these become permanent, and the whole contractile energy of the cell is then concentrated upon them. As the cytoplasm is pushed out farther and farther it becomes more and more limited in the direction of its movement. And when the pseudopodia are once fastened and fibrillation has begun it can move only in one direction, along the axis of the pseudopodia.

The energy which is at first expended in different directions, some of them antagonistic to others, becomes concentrated along the axis of the pseudopodium. Hence the resultant cytoplasmic contractions gain as much in strength and rapidity as they lose in mobility, and become capable of producing what we call a muscular contraction. The initial contractions are noticeably weak, and the intervals between

them are longer than in later development, but they do not stop here.

5. The muscle-fibres thus formed increase both in size and contractile power through exercise.

When first formed the fibres are simply fibrillated pseudopodia of single mesenchyme cells, hence all their substance must come from this one cell. And yet there is a perceptible increase in the size of the fibres, even after the entire body of the cell has gone out into them. This increase is most rapid and hence most apparent in those muscles which are most often contracted, e. g. the apical and circumoral muscles.

The increase in strength is admirably shown in the lappet muscles, each of whose single fibres scarcely shortens at all in its initial contractions. But after several days' development it can contract so vigorously as not merely to shorten, but also to assume the characteristic corkscrew shape.

6. Fibrillation and the consequent change from potential to kinetic contractility occurs only after the pseudopodium has attached itself to something outside the cell.

We found the same thing true of the spin-threads sent out by the polar bodies and the segmenting blastomeres.

The simplest explanation of this fact is that the constant movement of the cytoplasm in pushing out the spin-thread prevents contraction, but as soon as spin-thread or pseudopodium becomes attached the flow ceases, fibrillæ appear, and contraction ensues.

It is worthy of note that the nature of the contractions depends somewhat on the kind of attachment. When attached to a cell of the pilidium wall the contractions in the resultant muscle-fibre are like those of ordinary muscle. When threads from two cells anastomose without becoming attached to anything else, they contract in rhythmic pulsations like cardiac muscle.

This rhythm is shown even better in the muscles formed from mesenchyme cells in the veliger larvæ of certain nudi-

branch molluscs, and has been described elsewhere (49). Finally, in those cells where the cytoplasm becomes fibrillar without forming any connection at all outside the cell, as in ciliated epithelium, the contractions are in the form of rhythmic vibrations.

Sometimes it happens that the pseudopodia from two cells which have anastomosed, and have been contracting in rhythmic pulsations, become attached later to the pilidium wall. In such a case the nature of the contractions also changes, and they cease to be rhythmic, and become in all respects like ordinary muscle.

Histology.

Methods.—A pilidium larva is nearly as hostile to preservation as the medusa to which it is often compared. After a trial of many different preservatives the best was found to be platinum chloride, used either alone or in equal parts with acetic acid. Corrosive acetic can also be recommended, but is exceptionally difficult to wash out of the tissues.

For staining, Delafield's hæmatoxylin, followed by eosin or Orange G, gives good differentiation.

Blastula.—A segmentation cavity appears very early, and increases until the blastula consists of but a single layer of cells surrounding this cavity. These cells are elongated at right angles to the surface of the blastula, and vary greatly in size and shape, so that they project unequally into the central cavity, making the inner surface very rough.

The cells at the inferior pole are longer and narrower than the superior ones, so that there is thus early a differentiation into ectoderm and entoderm. The nuclei are spherical, comparatively large and uniformly granular; they are nearly always excentric in position, being much nearer the external end of the cell. The cytoplasm contains so much yolk material as to be opaque at first, but clears rapidly and becomes transparent enough to show the central cavity before gastrulation (fig. 55).

This cavity contains one or more loose cells which have been given off from the entoderm cells where they join the ectoderm. The loose cells are mesenchyme, and so far as observed none of them originate from the ectoderm.

Gastrula.—As soon as the cells begin to multiply for invagination and the formation of the intestine they become equal in size, and their inner ends are evened. The mesenchyme still continues to come off from the entoderm, and the latter is now clearly differentiated from the ectoderm (fig. 69).

The ectoderm consists of a single layer of epithelium. On the aboral surface it is a pavement epithelium made up of cubical cells, which are small at the superior pole, grow larger toward the equator, and then grow smaller near the border of the oral surface. By a comparison of different stages we find that the cells around the apical plate are the first to become transparent (fig. 70). Large clear spaces like vacuoles appear in these cells; but they are not empty spaces, for they react to stains in the same way as the gelatine which fills the body-cavity. These spaces gradually extend down toward the oral surface, until by the time the lappets are formed they can be found in every cell of the aboral ectoderm (fig. 73). In individual cells they are at first spherical, but quickly elongate parallel with the ectoderm surface. Then together with the cells they increase in area at the expense of their thickness, leaving the nuclei embedded in cytoplasm on the inner border of the cells.

The oral ectoderm is much thicker, and composed of cylindrical cells in which no clear spaces can be seen. These cells have larger nuclei than those in the aboral ectoderm, and are more active. From them are developed the lappets, the cilia row which surrounds the edge of both oral surface and lappets, and the invaginations which form the amnion and the ectoderm of the adult Nemertean (**13**).

The entoderm is much thicker than the ectoderm, but consists of a single layer of cells, cylindrical in form and similar to those of the oral ectoderm. They are more irregular in shape and overlap somewhat, so that they give

in some sections the appearance of a double layer. They retain their large blastula nuclei, and are quite active; they also remain opaque, with the exception of those on the anterior wall of the œsophagus, which are cleared in a different manner.

The Pilidium.

The aboral ectoderm, which was a pavement epithelium in the gastrula, has now become typically squamous, the cells being strongly flattened. As the cells increase in superficial area they lose the rounded form of the gastrula and become more and more angular, until they finally assume an irregular four- to six-sided form. The number of the cells remains practically constant, except at the border of the oral surface.

These facts, compared with the increase of clear areas in the gastrula, show that the pilidium grows largely, if not wholly, by a flattening of the aboral cells. The aboral "umbrella," as it is called, is simply a protective covering for the young embryo, to be thrown away at the time of metamorphosis. Hence its cells, when once formed, remain until final dissolution, simply flattening more and more to give the necessary increase in size. Sometimes the flattening is carried so far in the old pilidium that the body of the cell becomes thinner than the nucleus, and the latter bulges out into the body-cavity. Meanwhile the clear spaces have increased until they occupy nearly all the cell, leaving but little cytoplasm around the nucleus (fig. 72). The thinning and clearing of the ectoderm, combined with the "glass-clear" gelatine between ectoderm and entoderm, gives the pilidium great transparency. But we should not expect the individual cells to be held together very firmly, since they are in contact only along their thin edges, and hence they pull asunder easily under irritation.

But the oral ectoderm takes an active part in the development of the future Nemertean, and it is the only ectoderm except the apical plate that shows karyokinetic figures.

Its cells are smaller, but the nuclei are about the same size, and not being flattened the cells are held together more securely.

Both oral and aboral ectoderm are thickly covered with fine cilia, which are longer and stouter at the apical plate and the cilia row.

The apical plate is an invaginated thickening of the ectoderm. Metschnikoff regarded it "als eine Art indifferentes Gehirn," and the fibres going from it as a nerve commissure (**36**).

Bütschli later maintained that the fibres must be regarded as muscle, and that it was doubtful whether this plate really was the central organ of the nervous system (**11**).

Salensky considered Metschnikoff's assumption probable from a morphological standpoint, since the apical plate can be regarded as the homologue of that formed in the trochophore larva. But he added that it was unlike this in its simpler form, and it takes no part in the formation of the brain, and hence must be regarded as a rudimentary plate (**45**). He then gives the first, and, as far as known, the last account of the histological structure of the plate, closing with these words: "The question as to the nature of the bundle (of fibres connected with the plate) must be settled by a study of the development history of the plate and the fibres." We have just given the development history of the fibres, and have found that they are undoubtedly muscle. The plate originates in two or more cells found near the apical pole in a median section of a blastula. These cells stain more deeply than the others, and occasionally show karyokinetic figures (fig. 69); otherwise they are like the remaining ectoderm cells. But they divide and subdivide actively at right angles to the surface of the blastula and form a group of cells, one layer thick, a little anterior to the pole on the median line.

The cells are cylindrical in form, and of the same diameter throughout. Their nuclei are small at first, but become relatively large as the cells diminish, and are always situated close to the inner end of the cell. Their cytoplasm is more

compact, and hence not quite as transparent as the rest of the ectoderm. These plate cells divide slowly but steadily, while the surrounding ectoderm cells do not divide at all; hence they are pressed inward as an invagination into the body-cavity. Neither this invagination nor that of the intestine is quite symmetrical, the former being inclined toward the anterior end of the pilidium, and the latter toward the posterior end. As they invaginate the plate cells become smaller and more conical or wedge-shaped, the bases pointing inward toward the body-cavity (fig. 70). These changes proceed slowly, so that for two or three days the plate projects but slightly into the central cavity as a rounded swelling (fig. 71). But it grows more and more convex, until it acquires a thimble or nipple shape by the sixth day.

In preserved specimens the apical muscle is usually contracted, and the thimble is pulled out into a cone, and by comparing longitudinal with transverse sections it can be seen that the cone is nearly symmetrical in outline (cf. figs. 72 and 73).

A median section shows that the plate is composed of small, crowded conical cells, whose bases show through the ectoderm as distinct circles (fig. 74). The cells are shorter around the margin and longer toward the centre, each containing a large spindle-shaped nucleus and sometimes a highly refractive nucleolus.

Both cells and nuclei stain more deeply than the adjacent ectoderm, showing a corresponding difference in their activity.

The outer ends of the nuclei are usually pointed, and sometimes prolonged into a short, slender, thread-like process (fig. 76); but the inner ends are not so prolonged as Salensky has figured for Lineus (**45**), they are well rounded and plump.

A muscle fibril connects with the inner end of each of the cells near the centre of the plate, but not with those toward the margin. These fibrils can be seen to enter the plate through the structureless membrane which covers the whole plate, and are lost sight of in the substance of the cytoplasm.

If Salensky's figure be compared with this one of Cerebratulus (fig. 76) it will be noticed that in his figure (**45**, fig. 8) the plate is pulled in so far that fully one half the depression is formed of aboral ectoderm. Whether this is a normal condition in Lineus I am unable to say, but I have found a similar condition in Cerebratulus only when the apical muscle was strongly contracted. If this were true in Lineus the separate fibrils of the apical muscle when contracted would naturally pull out the inner ends of the cells to which they were attached, and may have produced the apparent prolongation.

The long stout cilia arise in bunches of four to six, from near the centre of the outer ends of the cells (fig. 76). Near the border of the plate they are finer and shorter than at the centre.

In life they are gathered into a compact bundle, looking like a single large flagellum, but in preserved specimens they are always separated and usually broken (cf. figs. 10 and 74).

Both surfaces of the plate are covered by a fine structureless membrane, through which cilia and muscle fibrils pass.

There are usually in the angle between the plate and the ectoderm mesenchyme cells whose function is unknown. They are yellowish or sometimes dark brown in colour in the living larva, and no processes can be detected coming from them. It is possible that they may be of the same nature as the so-called "eye-spots" of annelid larvæ, but this could not be determined.

The plate, therefore, is developed from ectoderm cells, which are smaller in size, less transparent, bear longer and more sensitive cilia, and whose nuclei are relatively larger, but which are otherwise undifferentiated from the remaining ectoderm.

None of these things necessarily indicate the formation of anything like nervous tissue; and, in view of the fact that the fibres connected with the plate are undoubtedly non-nervous in origin, structure, and function, we must conclude

that in Cerebratulus there is no nervous tissue in or connected with the apical plate. This plate differs from its morphological homologue in annelid larvæ in being made of a single layer of undifferentiated ectoderm instead of containing two layers more or less modified. In the trochophore the plate contains nervous tissue, which afterwards develops into the supra-œsophageal ganglion of the adult; in the Cerebratulus pilidium the plate is thrown away with the rest of the aboral ectoderm during metamorphosis, and we find no trace of any nerves at all.

The Cilia Rows.—These are rows of large cilia running along the border of the oral surface and around the edge of the lappets, and they are the chief organs of locomotion.

We can distinguish ectoderm wall cells, cilia cells, and a well-developed locomotor muscle system (fig. 75).

The ectoderm cells are similar in structure to other ectoderm, but increase in thickness as they approach the cilia cells, those on the aboral surface being thicker than the oral. Hence the upper border overhangs the under one, and acts as an organ of protection to the cilia, as noted by Salensky (**45**).

The cilia cells are smaller than the wall cells, and are arranged in three rows between the latter along the extreme edge of the lappets. They are wedge-shaped or pyramidal, with their apices turned inward. Their cytoplasm and nuclei are similar to those of the apical plate, and they are also covered on the exterior with a structureless membrane, through which the cilia pass. The latter are distributed over the entire surface of the three rows of cells, but show a tendency to gather in bundles. On the inner surface of the cilia cells, and between the wall cells, lie the locomotor muscles (fig. 77). These consist of a strong muscle band and of scattered mesenchyme cells. The band is made up of stout parallel fibres, which originate in the mesenchyme cells and terminate in the cilia cells (fig. 79). It is continuous with the circumoral muscle both anteriorly and posteriorly (fig. 77), and stains deeply with hæmatoxylin. It thus corresponds

exactly with the ring of muscle fibres and cells which lie at the base of the ciliated band in the trochophore larvæ.

The cells are small, and look like micromesencytes, except that they are mostly unipolar, the single pole giving off a long fibrous process. The row of cells can be followed from the lappets around the border of the body, but the muscle band blends with the circumoral muscle, and loses its individuality.

This locomotor muscle lies in exactly the position assigned by Salensky to the "nerve-ring;" fig. 79 is almost identical with fig. 4 of his description. But we have called them muscle for the following reasons. Salensky states that "the nerve-ring passes along the cilia row, and thus becomes the homologue of the nerve-ring in the annelid larvæ described by Kleinenberg." But he fails to note that Kleinenberg also describes a ring of muscle cells at the base of the ciliated cells, which is used by the larva in locomotion (**29**).

If either of these sets of locomotor apparatus are to be developed in so primitive a larva as the pilidium at the expense of the other set, the presumption is strongly in favour of the muscle. We would scarcely expect to find the nerve well developed, as Salensky claims, and no trace whatever of the muscle.

Again, borax carmine, orange G, and hæmatoxylin stain these fibres exactly the same as the other pilidium muscles, and no difference is perceptible in their structure or texture.

Another proof is that the cells whence the fibres originate are exactly like those from which the radial fibres arise.

It has never been doubted that the latter are anything but muscle cells; they, too, are unipolar or multipolar, as Salensky claims for the "nerve-cells," and his argument from form would apply equally well to both. Finally, this strand is continuous with the circumoral muscle (fig. 77), and if one is nerve, the other must be also; but if we concede that the circumoral ring is muscle, then the strand must also be muscle.

These facts, no one of which, perhaps, is sufficient alone,

when taken together furnish strong evidence that the fibres are muscle. Whether the strand also contains nerve-fibres must remain an open question; none can be seen, and pilidiums kept for days in water impregnated with methylene blue showed no trace of coloration.

Anteriorly the cilia rows, in passing from the lappets to the oral surface, invaginate in a large loop (fig. 82), which projects into the central cavity in the same manner as the apical plate.

The two loops are composed of the same sort of cells as the rest of the cilia rows, and appear undifferentiated. They appear about the sixth day, and increase in size up to the eighth or ninth day, after which they remain unchanged as long as I have succeeded in keeping any larvæ—about six weeks.

I believe these to be the first pair of invaginations which are to form the adult Nemertean, but, of course, have been unable to prove the matter.

The Intestinal Canal.—In the mature pilidium this is composed of two parts, each consisting of a single layer, and well differentiated. At first the intestine is composed throughout of large cylindrical cells, but as soon as the inner end begins to turn down posteriorly the constituent cells begin to differentiate. Those on the anterior and lateral walls of the œsophagus flatten out, increase in superficial area, and become transparent in a very similar manner to the aboral ectoderm. But the process is not carried so far, and the cells never lose their vitality. The flattening is not uniform, so that the cells grow thinner from the stomach toward the mouth, where they pass insensibly into the oral ectoderm.

The posterior wall of the œsophagus is thicker, its cells are smaller in area, more cylindrical in form, and do not become as transparent. They are also depressed along the mid-line into a shallow groove (fig. 82), which forms a channel for the food particles.

The stomach wall is thicker than that of the œsophagus, its component cells are larger and less transparent, and are

of two kinds, distinguished by the intensity with which they are stained.

Wall-cells.—These are more numerous, are prismatic in shape, and contain smaller nuclei. They do not stain very deeply, and are evidently the true entoderm.

Gland-cells.—Between the wall-cells are others scattered over the stomach, but most numerous at its anterior end (fig. 80). They are larger than the wall-cells, and are flask-shaped, with the narrow neck of the flask pointing inward, while the rounded body contains a large nucleus. They stain very deeply, and are evidently gland-cells as first stated by Metschnikoff (**33**) and later by Bürger (**13**), and not nerve-cells as claimed by Salensky (**45**).

The inner surface of both stomach and œsophagus is covered thickly with fine cilia, which are slightly longer in the stomach than in the œsophagus. At the base of the cilia is another structureless membrane, covering the whole interior of the intestine.

At the junction of stomach and intestine is the large sphincter muscle already described. It allows free passage of food particles when relaxed, but closes the opening entirely when contracted. Since the muscles are usually contracted in preserved specimens, it often happens that sections are obtained which appear similar to those given by Hubrecht (**25**); but watching the living larva for a few moments is sufficient to do away entirely with any idea that the stomach ends blindly at both ends. At about the sixth day a swelling is noted on the superior wall of the stomach posterior to the pyloric valve. This develops into a shallow evagination whose walls are made up of cells smaller and more crowded than the adjacent stomach entoderm. But this evagination was carried no further in the oldest pilidiums reared, and so its ultimate purpose could not be determined.

Summary.—1. A segmentation cavity appears very early, and increases until the blastula consists of a single layer of cells elongated at right angles to the surface and surrounding this cavity.

2. The ectoderm is a one-layered pavement epithelium, made up of cubical cells on the aboral surface and cylindrical cells on the oral surface. The entoderm is also one-layered and made up of cylindrical cells, but it is thicker and more opaque than the ectoderm, and its cells overlap considerably.

3. The aboral ectoderm remains constant after being formed, and the increase in size is accomplished by a strong flattening without cell division. This, together with a gradual clearing of the cell protoplasm, gives the pilidium great transparency.

4. The apical plate is an invaginated thickening of the ectoderm, made up of conical cells whose bases turn inward toward the body-cavity. The nuclei are pointed and sometimes prolonged into thread-like processes on the outer ends, but are rounded and plump on the inner ends. The component fibrils of the apical muscle connect with the inner ends of all cells near the centre of the plate.

5. The long stout cilia which form the apical tuft arise in bunches of four to six from the centre of the inner ends of the cells, and in life are gathered into a central bunch which looks like a single large flagellum.

6. The cilia cells, whence arise the large cilia running along the border of the oral surface and around the edge of the lappets, are arranged in three rows. The cilia themselves are given off in bunches from the outer ends of the cells.

7. Just inside the cilia cells is a strong band of locomotor muscle-fibres and scattered mesenchyme cells. A careful study of their origin and development, the development of the plate itself, its histological structure and staining, all go to prove that both fibres and cells are muscle, and that there is no nervous tissue in the plate or connected with it.

8. The anterior wall of the œsophagus becomes partially flattened and cleared in the same manner as the aboral ectoderm, but the posterior wall retains its original thickness. It is grooved along the mid-line to form a channel for the food.

9. The stomach walls contain numerous gland-cells, which are flask-shaped, with the neck of the flask pointing inward. The inner surface of both stomach and intestine is covered with cilia, and at their junction is a large sphincter muscle which controls the passage of the food particles and waste material.

Addenda.

Since the foregoing went to the printer I have received an article entitled "Development of the Pilidium of Certain Nemerteans," by W. R. Coe.

This excellent paper discusses briefly the segmentation, gastrulation, and pilidium stages in Micrura cæca, Cerebratulus Leidyi, and C. marginatus.

Together with the present paper it was practically finished before either author became aware that the other was working upon the subject. The close agreement in the results obtained is all the more gratifying in view of their entire independence.

I wish briefly to mention the following points:

1. In segmentation C. lacteus transposes the second and third cleavage planes of the other three species, making the second horizontal and the third vertical. Otherwise the four agree in all essential features of segmentation and gastrulation.

2. While Coe has not given the development of the apical plate and the muscular system in detail, it is evident from his drawings that this development in both cases is almost identical with that here given. The arrangement of the parietal muscles, especially in Micrura cæca, is somewhat different from that in C. lacteus, as might be expected. But the mesenchyme cells from which they are formed are evidently smaller than those which form the other muscles, and correspond to the micromesencytes in C. lacteus.

3. The statements in reference to the presence of nervous tissue are wisely cautious, but it is easy to see that Coe does

not put much faith in Salensky's interpretation (**45**). He distinctly states (p. 253) that the so-called "nerve-cells" in the entoderm "are obviously nothing but gland-cells, which are partially filled with a deeply staining secretion and have no sensory function whatever."

In all four of these species, without exception, the fibres connecting the apical plate with the oral ectoderm are muscular in origin. Also the elements which Salensky describes as nerve-cells and nerve-fibres are found to stain easily with common muscle stains, while they do not differentiate at all with methylene blue or any other distinctive nerve stain. This should make us somewhat cautious in accepting the apical plate of the pilidium as an exact homologue of that in the trochophore larva of Annelids.

Bibliography.

An excellent bibliography will be found in Bürger's monograph (**13**), which contains a brief abstract of every paper mentioned. Only those papers are mentioned here to which actual reference is made in the text.

1. Agassiz, A.—"The Embryology of Autolytus cornutus," 'Boston Journ. Nat. Hist.,' vol. vii, 1863.
2. Andrews, E. A.—"Activities of the Polar Bodies of Cerebratulus," 'Archiv f. Entwick.,' Bd. vi, Heft 2, 1898.
3. Andrews, E. A.—"Filose Activities in Metazoan Eggs," 'Zool. Bulletin,' vol. ii, No. 1, 1898.
4. Andrews, G. F.—"The Living Substance as such, and as Organism," 'Journ. Morph.,' vol. xii, 1897.
5. Andrews, G. F.—"Some Spinning Activities of Protoplasm in Star-fish and Echinus Eggs," ibid.
6. Beattie.—"On the Reproduction of Nemertes Borlassii (Lineus longissimus)," 'Proc. Zool. Soc.,' London, Part xxvi, 1858.
7. Barrois, Jules.—"Mémoire sur l'embryologie des Némertes," 'Ann. Sc. Nat.' (6), tome 6, 1877.

8. van Beneden, M.—"Recherches sur la Faune littorale de Belgique," 'Mém. d. l'Acad. des Sc. Belg.,' tome xxxii, 1861.

9. van Beneden, E.—"Recherches sur la Maturation de l'Œuf, la fécondation et la division cellulaire," 'Archiv de Biol.,' tome iv, 1883.

10. Benham, W. B.—"Fission in Nemertines," 'Quart. Journ. Micr. Sci.,' vol. xxxix, 1896.

11. Bütschli, O.—"Einige Bemerkungen zur Metamorphose des Pilidium," 'Archiv für Naturgesch., 1873.

12. Bütschli, O.—"Vorläufige Mittheilungen über Untersuchungen betreffend die ersten Entwickelungsvorgänge im befruchteten Ei von Nematoden und Schnecken," 'Zeit. f. wiss. Zool.,' Bd. xxv, 1875.

13. Bürger, Otto.—"Die Nemertinen des Golfes von Neapel," 'Fauna u. Flora d. Golfes v. Neapel,' 22te Monographie, 1895.

14. Busch, W.—'Beobachtungen über Anatomie und Entwickelung einiger wirbellosen Seethiere,' 1851.

15. Coe, W. R.—"On the Anatomy of a Species of Nemertean (Cerebratulus lacteus, Verrill), with remarks on certain other species," 'Trans. Conn. Acad.,' vol. ix, 1895.

16. Coe, W. R.—"The Maturation and Fertilisation of the Egg of Cerebratulus," 'Zool. Jahrb.,' Bd. xii, 1899.

17. Conn, H. W.—"Life History of Thalassema," 'Stud. Biol. Lab. Johns Hopkins Univ.,' vol. iii, No. 7, 1886.

18. Desor, E.—"Embryology of Nemertes," 'Proc. Boston Nat. Hist. Soc.,' vol. vi, 1848.

19. Dieck, G.—"Entwickelungsgeschichte des Nemertinen," 'Jenaische Zeitschrift,' Bd. viii, 1874.

20. Englemann, T. W.—"Zur Anatomie und Physiologie der Flimmerzellen," 'Archiv ges. Phys.,' Bd. xxiii, 1880.

21. von Erlanger, R.—"Entwickelungsgeschichte des Nematoden," 'Biol. Centralb.,' Bd. xvii, Heft 4, 1898.

22. Gegenbaur, C.—"Bemerkungen über Pilidium gyrans, etc.," 'Zeit. f. wiss. Zool.,' Bd. v, 1854.

23. Gage, S. H.—"The Life History of the Toad," 'Teacher's Leaflet,' No. 9, Coll. Agric., Cornell Univ., 1898.

24. Heidenhain, M.—"Cytomechanische Studien," 'Archiv f. Entwick.,' Bd. i, Heft 4, 1895.

25. Hubrecht, A. A. W.—"Zur Embryologie der Nemertinen," 'Zool. Anz.,' 1885.

26. Hubrecht, A. A. W.—"Contributions to the Embryology of the Nemerteans," 'Quart. Journ. Micr. Sci.,' vol. xxvi, 1886.

27. Hubrecht, A. A. W.—"Report on the Nemertines," 'Reports of the Challenger Expedition,' 1887.

28. Krohn, J.—"Ueber Pilidium und Actinotrocha," 'Müller's Archiv,' 1858.

29. Kleinenberg, N.—"Sull' origine del sistema nervosa central degli Annelidi," 'Mem. Accad. Lincei Roma,' tome x, 1881.

30. Lee, A. B.—"La Spermatogenèse chez les Némertiens, à propos d'une théorie de Sabatier," 'Recueil zu Suisse,' tome iv, 1887.

31. Leuckart und Pagenstecher.—"Untersuchungen über niedere Seethiere," 'Müller's Archiv,' 1858.

32. Mark, E. L.—"Maturation, Fecundation, and Segmentation of Limax campestris," 'Bull. Mus. Comp. Zool.,' Harvard College, No. 6, 1881.

33. McIntosh, W. C.—"Notes on the Development of Lost Parts in the Nemerteans," 'Journ. Linn. Soc.,' vol. x, 1870.

34. McIntosh, W. C.—'Marine British Annelids, Nemertea,' 1873–4.

35. Metschnikoff, E.—"Studien über die Entwickelung der Echinodermen und Nemertinen," 'Mém. Acad. Imp. Pétersbourg,' series vii, tome xiv, No. 8, 1870.

36. Metschnikoff, E.—"Vergleichend embryologische Studien," 'Zeit. f. wiss. Zool.,' Bd. xxxvii, 1882.

37. Montgomery, T. H.—"On the Connective Tissues and Body-cavities of the Nemerteans, with Notes on Classification," Spengel's 'Zool. Jahrb.' (Anat. Abth.), Bd. x, 1897.

38. Müller, Joh.—"Pilidium gyrans," 'Archiv für Anat. und Phys.,' 1847.

39. Müller, Joh.—"Ueber verschiedene Formen von Seethieren," 'Müller's Archiv,' 1854.

40. Randolph, H.—"The Regeneration of the Tail in Lumbriculus," 'Zool. Anzeiger,' Jahrg. xiv, 1891.

41. Renier, St. A.—'Prospetto della Classe dei Vermi,' 1804.

42. Sabatier, A.—"La Spermatogenèse chez les Némertiens," 'Revue Sc. Nat. Montpellier' (3), tome ii, 1883.

43. Salensky, W.—"Zur Entwickelungsgeschichte der Borlasia vivipara," 'Biol. Centralb.,' Bd. ii, No. 24, 1883.

44. Salensky, W.—"Recherches sur le développement du Monopora vivipara," 'Archiv. de Biol.,' tome v, 1884.

45. Salensky, W.—"Bau und Metamorphose des Pilidiums," 'Zeit. f. wiss. Zool.,' Bd. xliii, 1886.

46. Verrill, A. E.—"The Marine Nemerteans of New England," 'Trans. Conn. Acad.,' vol. viii, 1892.

47. WATASÉ, S.—"Studies on Cephalopods: I. Cleavage of the Ovum," 'Journ. Morph.,' vol. iv, No. 3, 1891.

48. WHITMAN, C. O.—"The Embryology of Clepsine," 'Quart. Journ.' Micr. Sci.,' vol. xviii, 1878.

49. WILSON, C. B.—"Activities in Mesenchyme Cells of Certain Larvæ,' 'Zool. Bull.,' vol. ii, No. 1, 1898.

50. WILSON, E. B.—"Observations on the Early Developmental Stages of some Polychætous Annelids," 'Stud. Biol. Lab. Johns Hopkins Univ.,' vol. ii, 1882.

51. WILSON, E. B.—'The Cell in Development and Inheritance,' The Macmillan Company, New York, 1896.

52. ZIMMERMANN, K. W.—"Studien über Pigmentzellen, etc.," 'Archiv f. micros. Anat.,' Bd. xli, 1893.

EXPLANATION OF PLATES 9—11,

Illustrating Mr. Chas. B. Wilson's paper on "The Habits and Early Development of Cerebratulus lacteus (Verrill)."

KEY TO LETTERS.

am. Apical muscle. *ani.* Anterior invagination. *bv.* Blood-vessel. *c.* Intestinal cæca. *cc.* Cilia cells. *cm.* Circular muscles. *com.* Circumoral muscle. *ct.* Connective tissue. *dvm.* Dorso-ventral muscles. *ec.* Ectoderm. *en.* Entoderm. *gc.* Gelatine cell. *glc.* Gland-cell. *gr.* Groove for food. *im.* Interparietal muscle. *lam.* Lappet muscles. *lm.* Longitudinal muscles. *lom.* Locomotor muscles. *o.* Ovary. *œ.* Œsophagus. *pm.* Post-œsophageal muscle. *s.* Stomach. *sc.* Sperm mother-cell. *t.* Testis. *wc.* Wall-cell. *yc.* Yolk-cell.

PLATE 9.

FIG. 1.—Cerebratulus swallowing a Nereis five minutes after dismembering the posterior half of its body. Photograph from preserved specimen, one eighth life size.

FIG. 2.—Cerebratulus with regenerating papilla. Photograph from life, one eighth life size.

FIG. 3.—Male (left) and female (right) Cerebratulus, the latter showing normal anal papilla. Photograph from life, one eighth life size.

FIG. 4.—Cerebratulus with proboscis sheath cut open to show how the proboscis is coiled when withdrawn. Photograph from preserved specimen, one eighth life size.

All the remaining figures, with the exception of Nos. 11, 53, 54, and 59, have been drawn with a camera lucida on a Leitz microscope. The magnification is indicated by giving the Leitz numbers of the eye-piece and objective used. The figures have then been reduced one half in making the plates.

FIG. 5.—Gastrula, showing shape and arrangement of ectodermal cells and beginning of cilia rows. 3 and 7.

FIGS. 6 and 7.—Successive stages in the process of escaping from the egg membranes. 3 and 3.

FIG. 8.—Gastrula just escaped from the membranes, thirty-eight hours old. Cilia and flagella drawn the exact length seen. 1 and 7.

FIG. 9.—Larva forty-eight hours old, first stage in formation of apical muscle. 1 and 7; tube drawn 60 mm.

FIG. 10.—Same, fifty-four hours old, second stage; side view.

FIG. 11.—Same, ninety-six hours old, third stage. Zeiss camera lucida, magnified 575 diams.

FIG. 12.—Same, 108 hours old, fourth stage; end view. 1 and 7; tube drawn 60 mm.

FIG. 13.—Same, 120 hours old, fifth stage; side view, showing apical muscle attached to dorsal wall of œsophagus.

FIG. 14.—Same, six days old, sixth stage, showing apical muscle divided and extending down on either side of the intestine to fasten to the oral surface anterior to the mouth. This figure shows also interparietal, post-œsophageal, and circumoral muscles.

FIG. 15.—Same, ten days old, seventh stage. Apical muscle fully developed; post-œsophageal muscle forming a triangular sheet; radiating lappet muscles well developed.

FIG. 16.—Same, twelve days old; surface view showing parietal muscles.

FIG. 17.—Transverse interparietal muscle attached to apical plate, from larva six days old. 1 and 7.

FIG. 18.—Eggs taken from ripe ovary and examined before they have touched any water. 3 and 3.

FIG. 19.—Same after immersion in salt water three minutes.

FIG. 20.—A ripe, freshly laid ovum, unfertilised, showing membranes and attachment protuberance. 1 and 7.

Fig. 21.—Transverse section of a regenerating papilla very near its posterior end, showing ventral grooves and beginning of the lateral nerve-cords. 1 and 3.

Fig. 22.—Same farther forward, showing lateral nerve-cords migrating toward their normal position.

Fig. 23.—Same still farther forward; nerve-cords nearly in their normal position, and circular muscles well developed.

PLATE 10.

Fig. 23*a*.—Fertilised egg, showing flattening of the superior pole previous to the giving off of the polar bodies. 1 and 7.

Figs. 24—36.—Maturation of the egg and formation of the polar bodies, with the beginning of filose activities. 1 and 7, tube drawn 60 mm. These drawings are all from the same egg, and were taken at the following intervals:—11.05 a.m., 11.065, 11.08, 11.21, 11.216, 11.22, 11.228, 11.235, 11.24, 11.27, 11.28, 11.335, and 11.40.

Fig. 37.—A second egg, showing abnormal activities during completion of the polar bodies. 1 and 7; tube drawn 60 mm.

Fig. 38.—A third egg with unequal papillæ and slightly abnormal activities. Magnification the same.

Fig. 39.—Egg with polar bodies pressing against the inner membrane and bulging it outward. 1 and 7; tube drawn 60 mm.

Fig. 40.—Polar bodies of egg shown in Figs. 24—36, taken at 11.36. 3 and 7; tube drawn 60 mm.

Fig. 41.—Same, first polar body at 11.17.

Fig. 42.—Polar bodies and sperm at beginning of first segmentation. 3 and 7.

Fig. 43.—Same at close of first segmentation.

Fig. 44.—Polar bodies during 64-cell stage. 3 and 7; tube drawn 60 mm.

Fig. 45.—Same in 128-cell stage.

Figs. 46—50.—First segmentation, showing filose activities of polar bodies and blastomeres. All figures from the same egg at the following intervals:—12.06 m., 12.065, 12.09, 12.11, 12.16. 1 and 7; tube drawn 60 mm.

Fig. 51.—Egg in which blastomeres were almost entirely separated, and in which they showed perceptible motion during flattening. Same magnification.

Fig. 52.—Same egg as in Figs. 46—50, in the 4-cell stage (12.30). Central opening crossed by spin-threads.

Fig. 53.—Four-cell stage, top view. Enlarged from a camera lucida sketch of living egg.

Fig. 54.—Eight-cell stage, showing dextral twisting of the upper cells. Enlarged from camera lucida sketch of living egg.

Fig. 55.—Blastula with differentiated ectoderm and entoderm, and mesenchyme cell separating from the latter. 3 and 7, from a preserved and mounted specimen.

Fig. 56.—Section showing nuclear spindle parallel with surface subsequent to giving off of first polar body. 3 and 3.

Fig. 57.—Same with spindle diagonal after extrusion of second polar body.

Fig. 58.—Same with segmentation spindle for first segmentation.

Fig. 59.—Sperms, enlarged from camera lucida sketch, cf. Fig. 42.

PLATE 11.

Fig. 60.—Longitudinal horizontal section of a regenerating papilla, showing formation of sexual pouches and intestinal cæca; anus terminal. 3 and Tolles 1-inch objective.

Figs. 61 and 62.—Longitudinal horizontal sections of male and female; immature genital pouches scattered through the connective tissue. Killed in April. 1 and 3.

Fig. 63.—Transverse section of female killed in April, showing relation of developing egg-pouches to inner longitudinal muscle layer. 3 and 3.

Fig. 64.—Portion of immature ovary from Fig. 62, enlarged to show method of egg development. 1 and 7.

Fig. 65.—A single egg pouch of Fig. 62, enlarged to show egg cells, yolk cells, and glycerine cells. The walls are formed from connective tissue. 1 and 7.

Fig. 66.—A single sperm pouch of Fig. 61, enlarged to show formation of sperm mother-cells. The connective tissue here extends into the pouch, and the sperm cells are supported upon it. 3 and 3; tube drawn 60 mm.

Fig. 67.—A small portion of Fig. 66, enlarged to show the way in which the sperm cells are borne on the mesoderm strands. 3 and 7; tube drawn 60 mm.

Fig. 68.—A single sperm pouch magnified still farther to show transformation from sperm mother-cells into sperms. 3 and one twelfth oil immersion.

Figs. 69—73.—Vertical sections of a late blastula and four gastrulas in different stages of development, showing the origin and development of the apical plate, the formation of mesenchyme cells, and clearing of ectoderm. 1 and 7; tube drawn 60 mm.

Fig. 74.—Longitudinal vertical section of pilidium; apical plate fully developed with apical muscle attached; aboral ectoderm much flattened and perfectly transparent; oral ectoderm thicker, more opaque, and beginning to invaginate anteriorly; micromesencytes scattered over the inner surface of both ectoderm and entoderm. 1 and 7.

Fig. 75.—Transverse section of lappet; three rows of cells bearing long cilia; locomotor muscles. 3 and one twelfth oil immersion.

Fig. 76.—Longitudinal section of apical plate, showing arrangement of flagella in bunches and the connection of the muscle-fibres. 3 and one twelfth oil immersion.

Fig. 77.—Longitudinal vertical section through the lappet, the œsophagus wall, and the stomach. The locomotor muscle band (*lom.*) is here seen to be continuous with the circumoral muscle (*com.*). 3 and one twelfth oil immersion.

Fig. 78.—Longitudinal vertical section of the lappet through the wall cells of the cilia rows, with a portion of the locomotor muscle band and the mesenchyme cells forming the radial muscles of the lappets. 3 and one twelfth oil immersion.

Fig. 79.—Longitudinal vertical section as in Fig. 77, showing the locomotor muscle band composed of cells and fibres. 3 and one twelfth oil immersion.

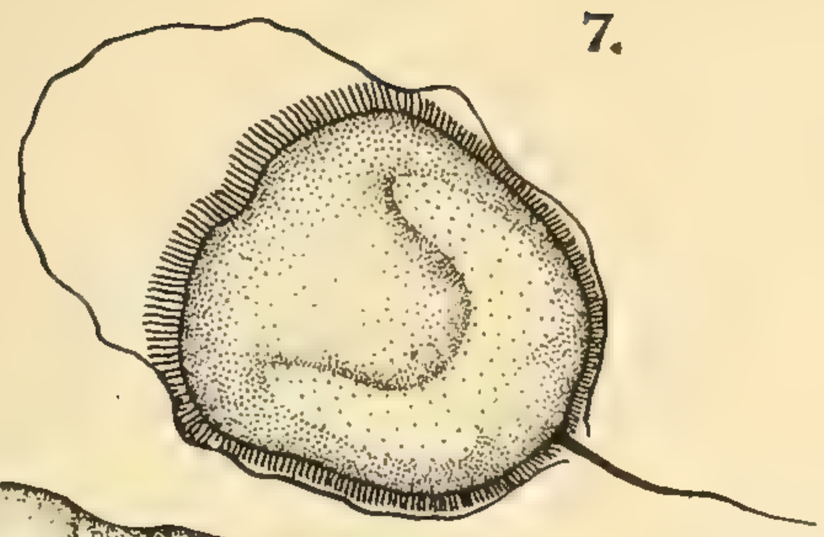
Fig. 80.—Longitudinal vertical section of stomach, showing ordinary entoderm and gland cells. 3 and one twelfth oil immersion.

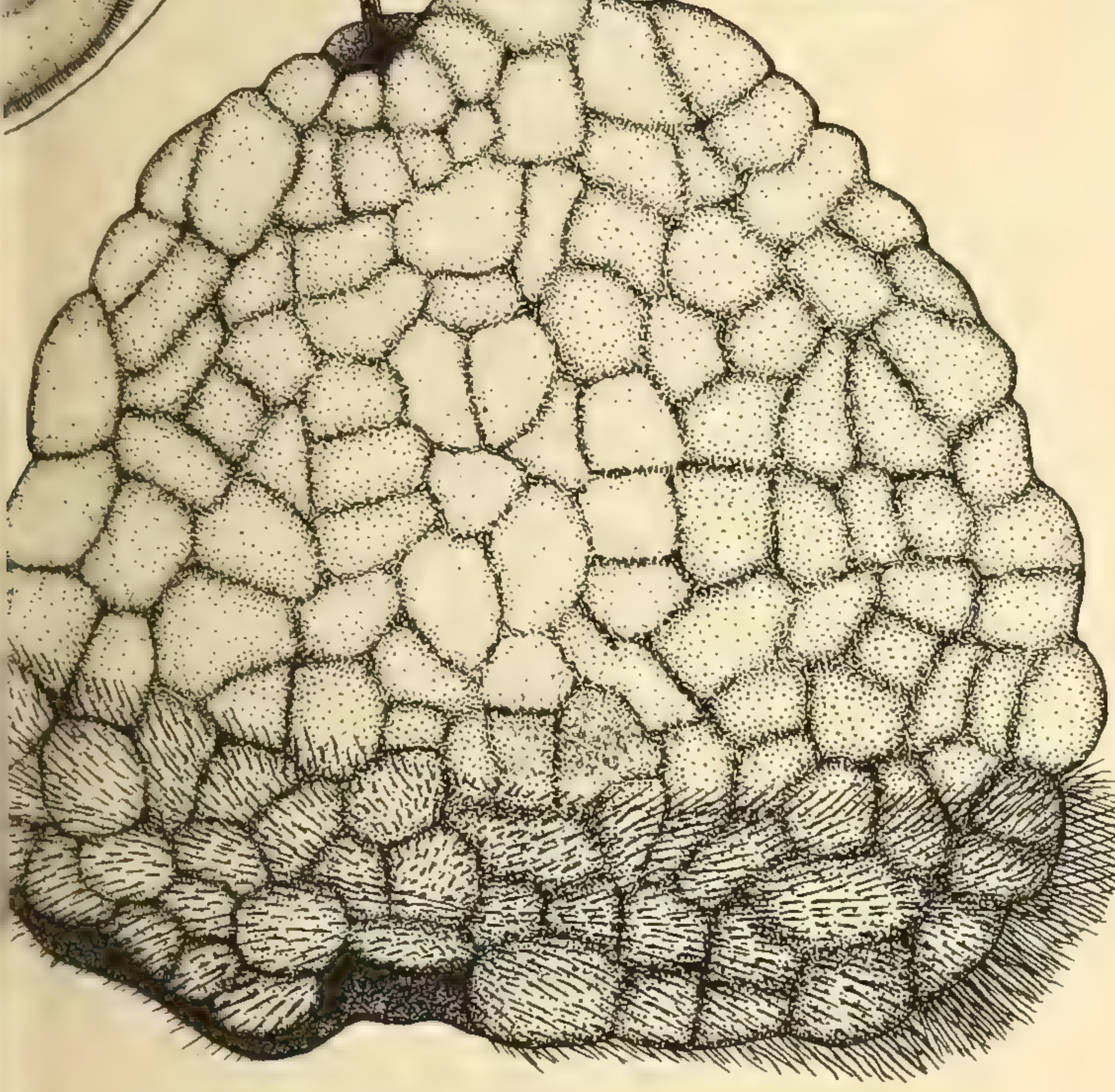
Fig. 81.—Same as Fig. 77, but showing more plainly the row of mesenchyme cells which form the radial lappet muscles.

Fig. 82.—Longitudinal horizontal section just above the mouth, showing the two anterior invaginations with mesoderm attached; the walls of the œsophagus with a central groove posteriorly for the food particles. 3 and 8; tube drawn 60 mm.

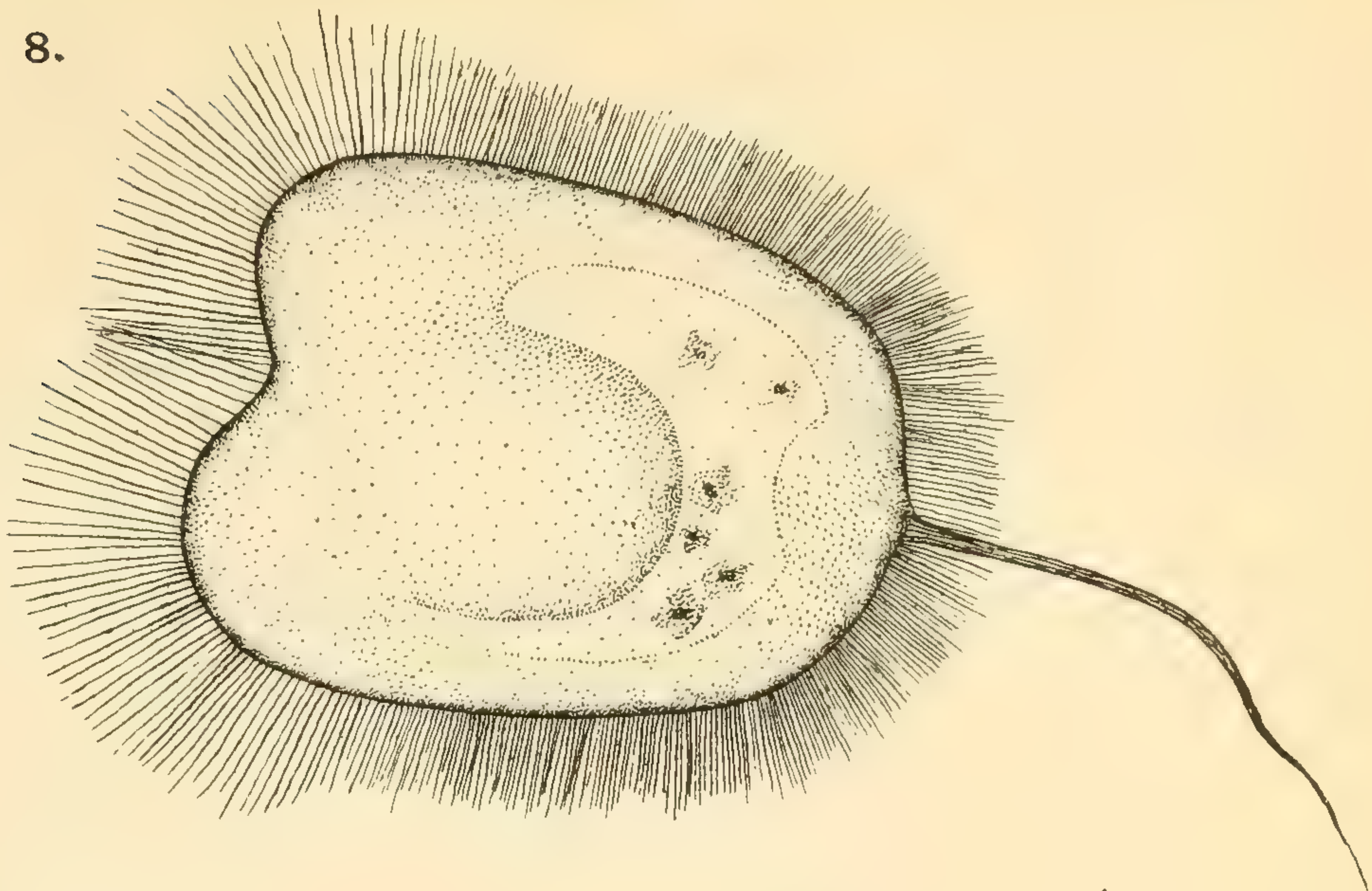
C.H.Wilson del.

7.

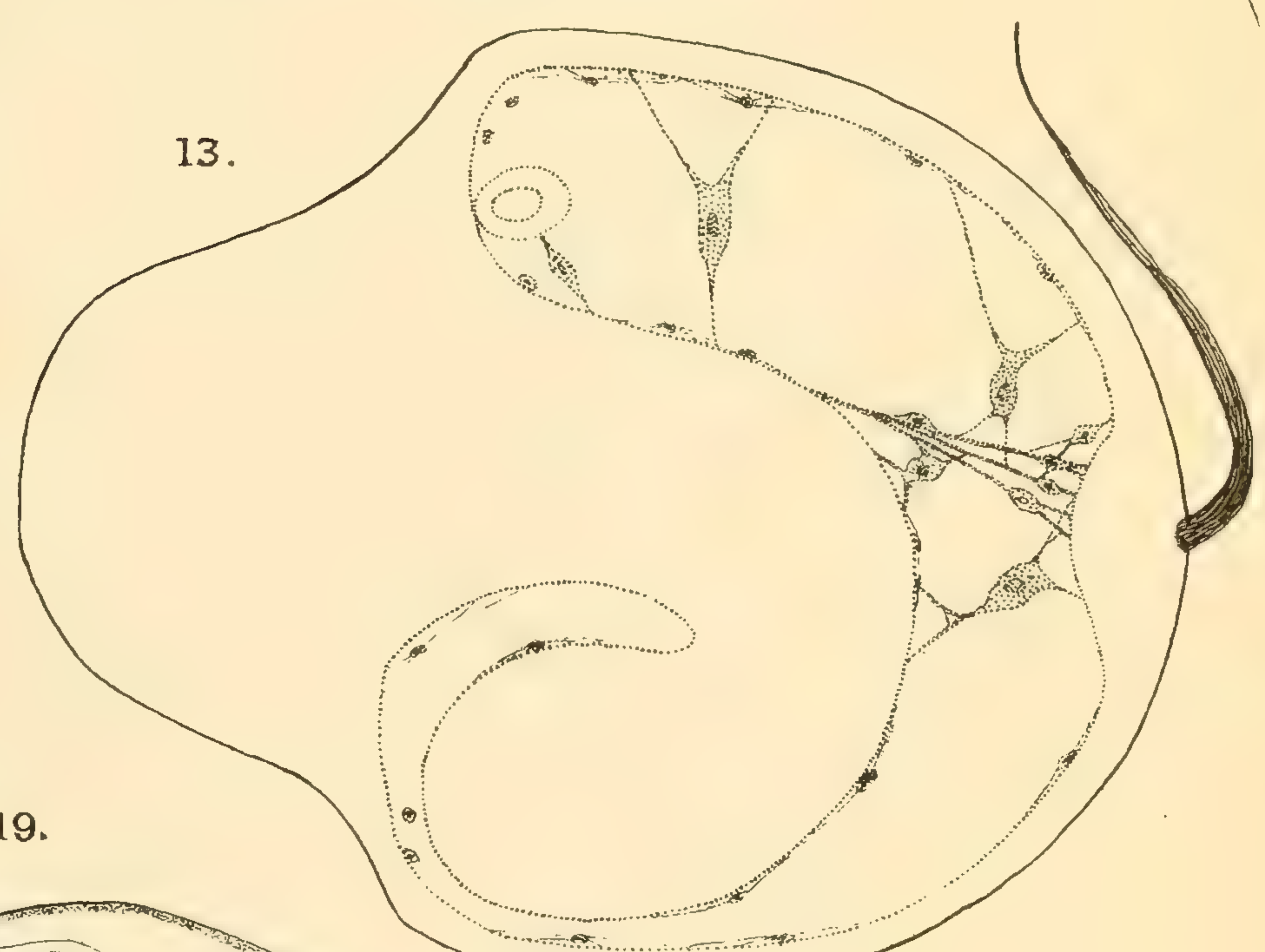


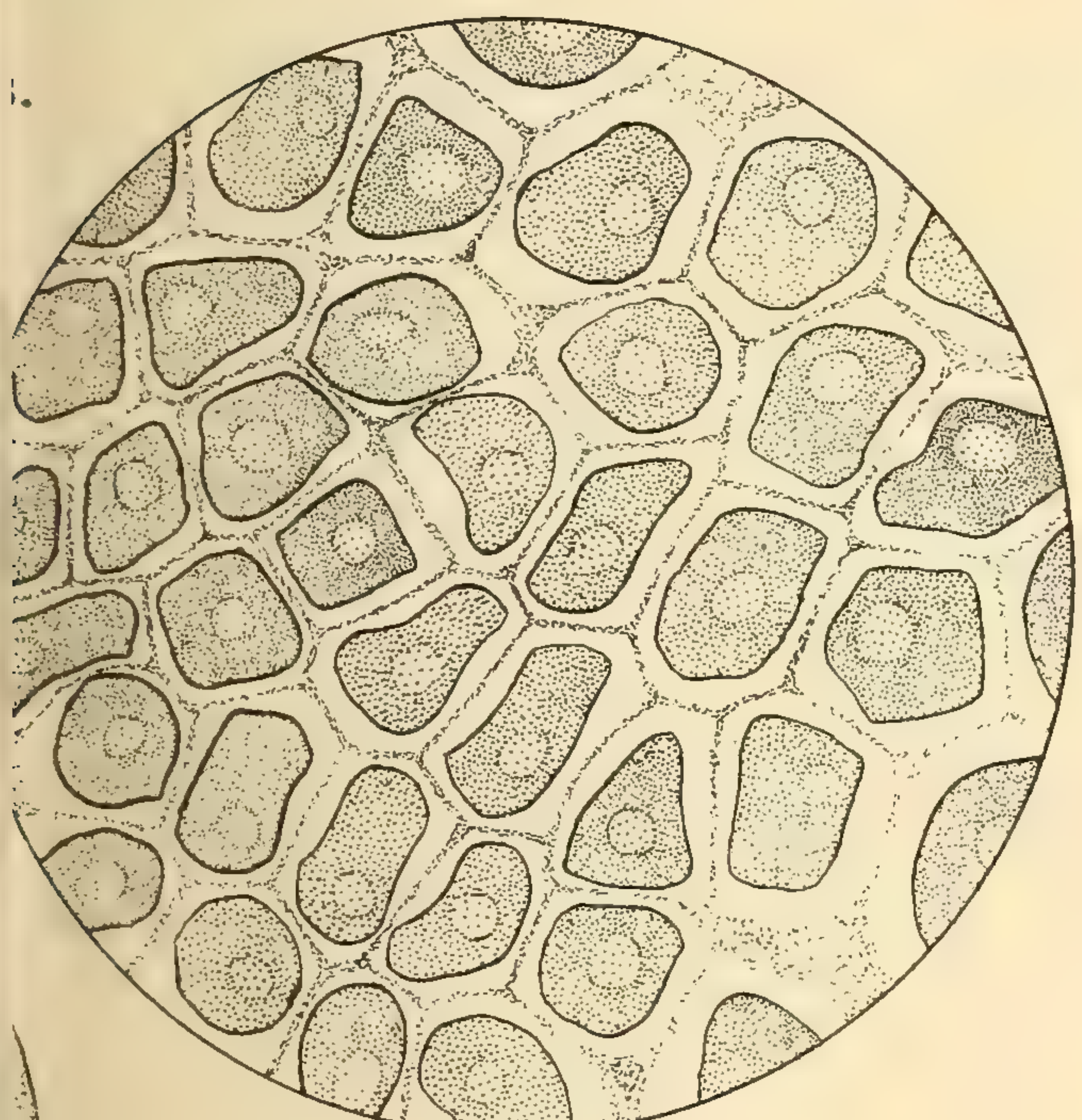


8.

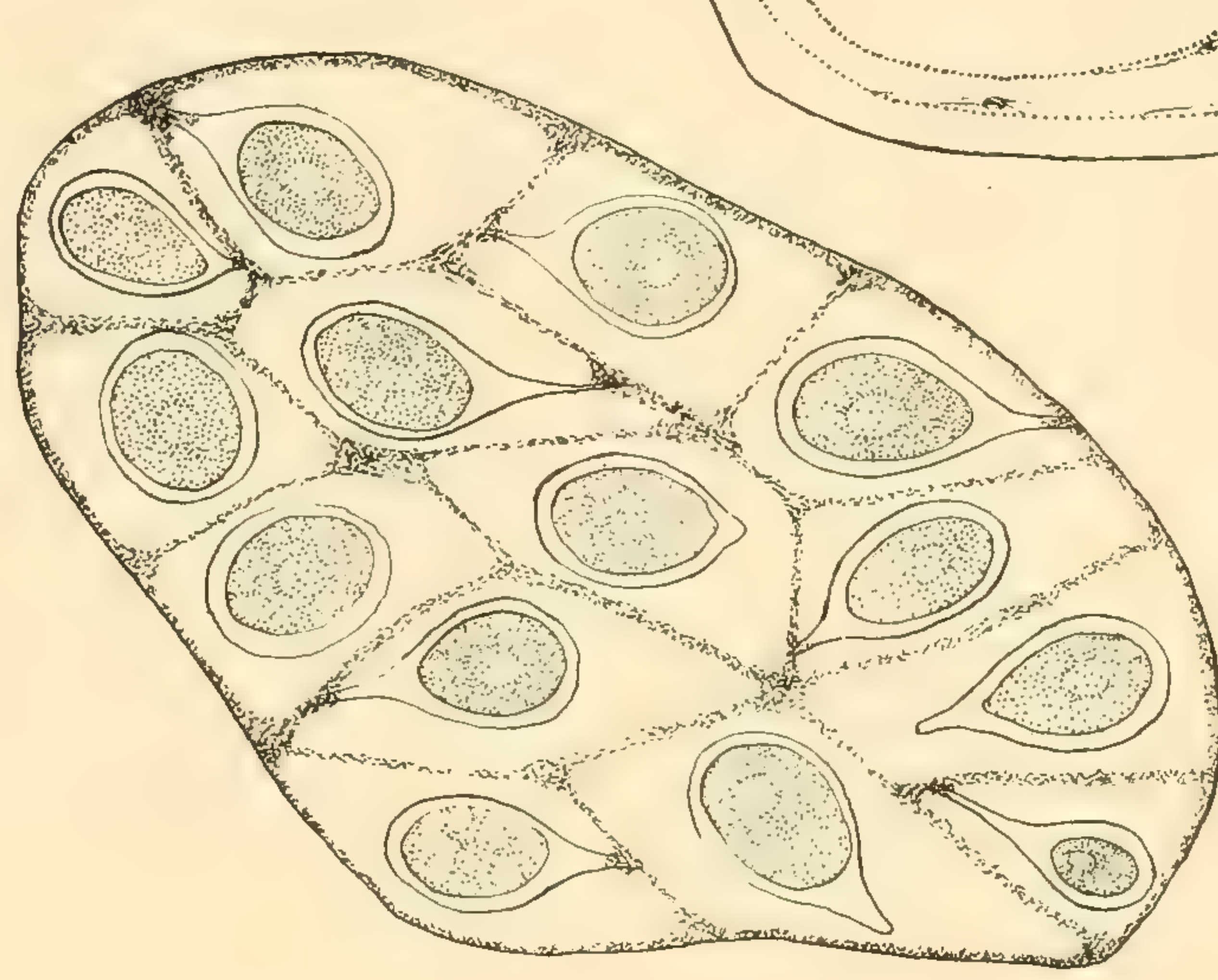


13.

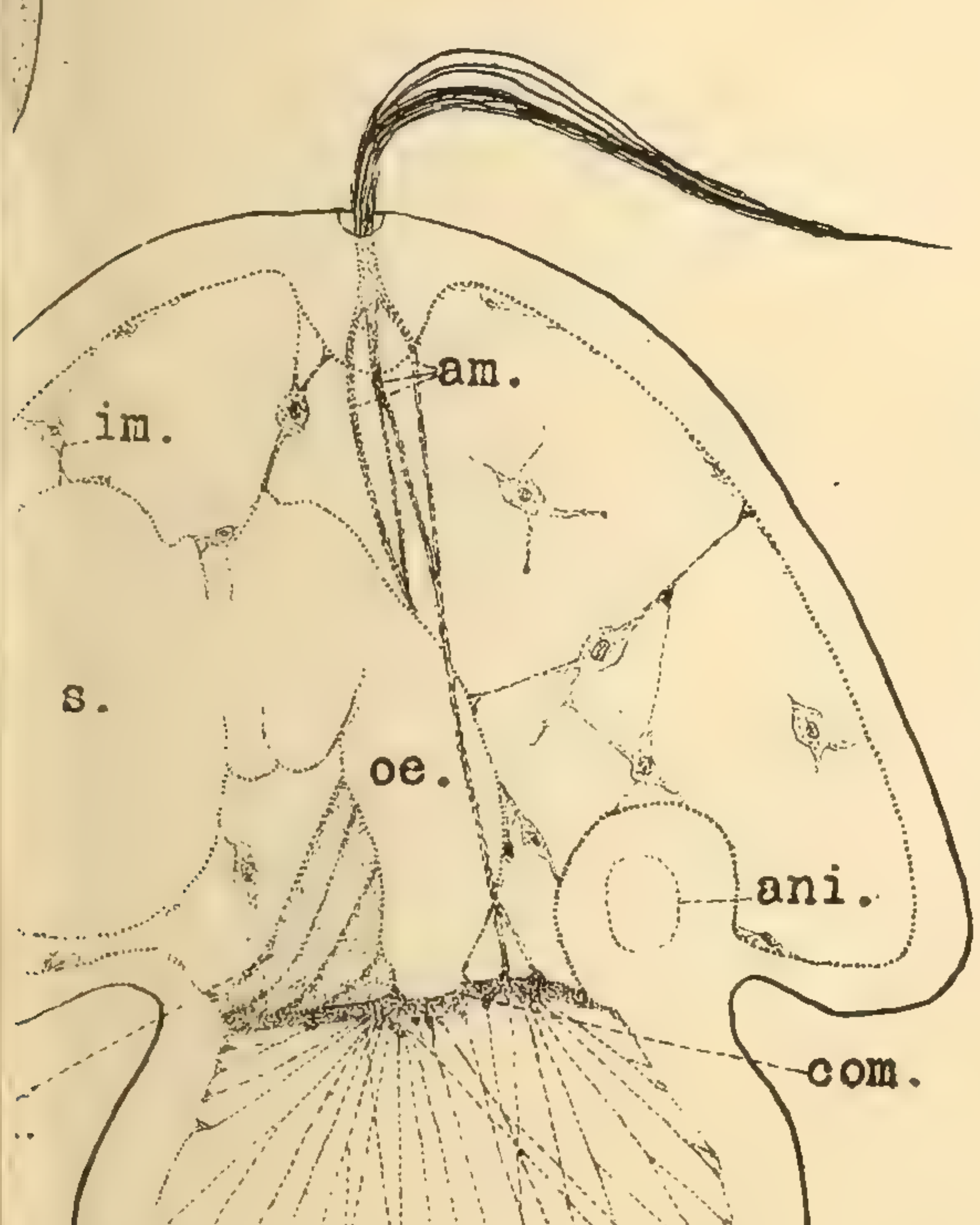




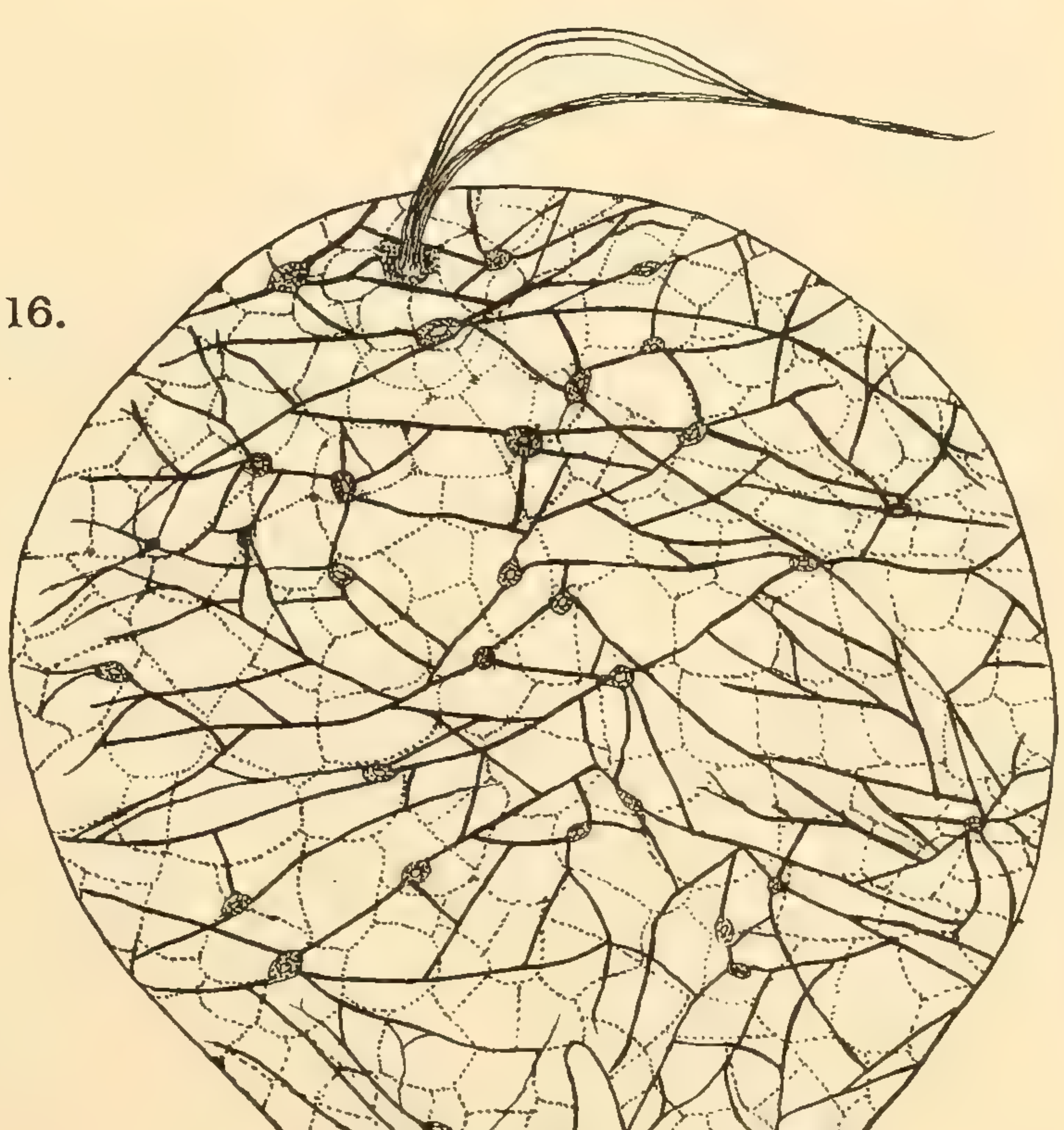
19.



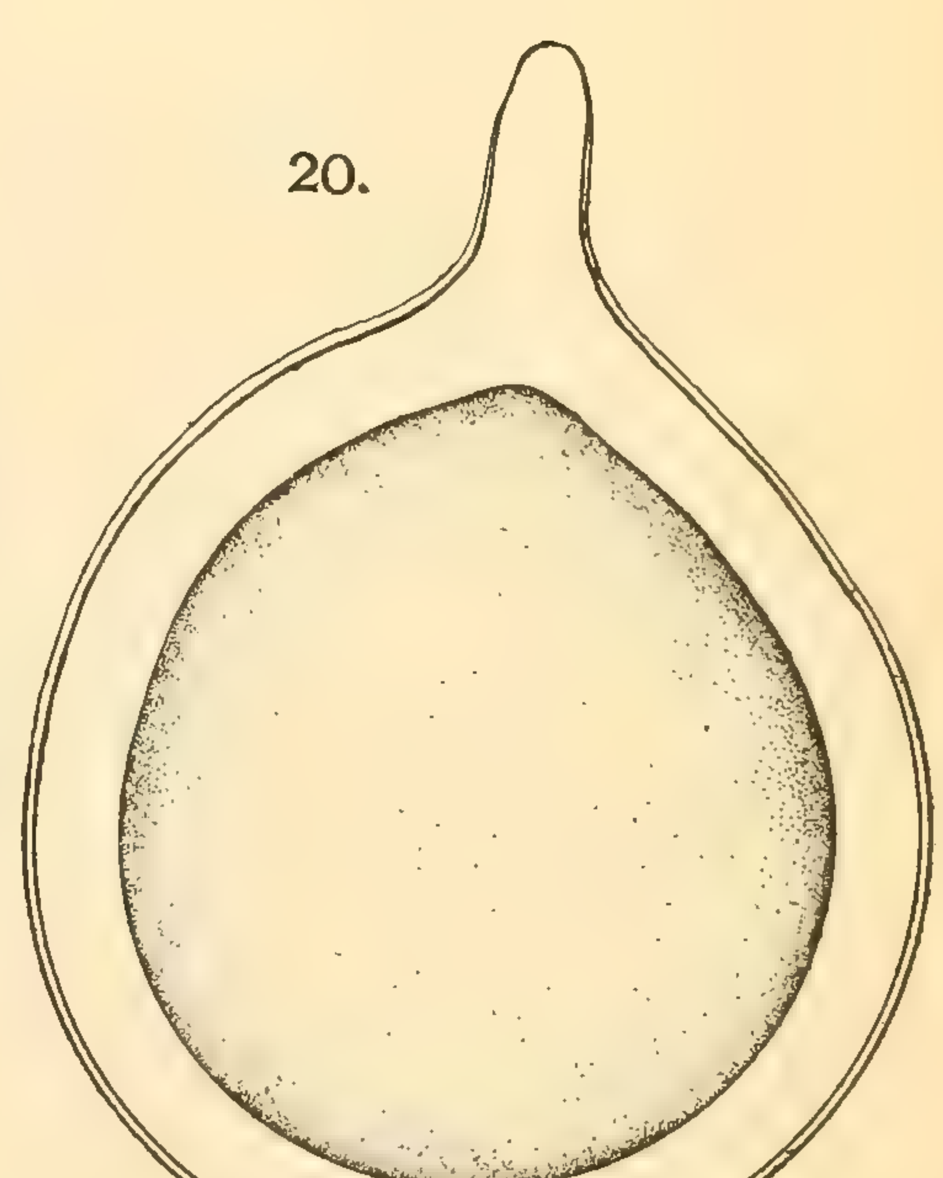
23.

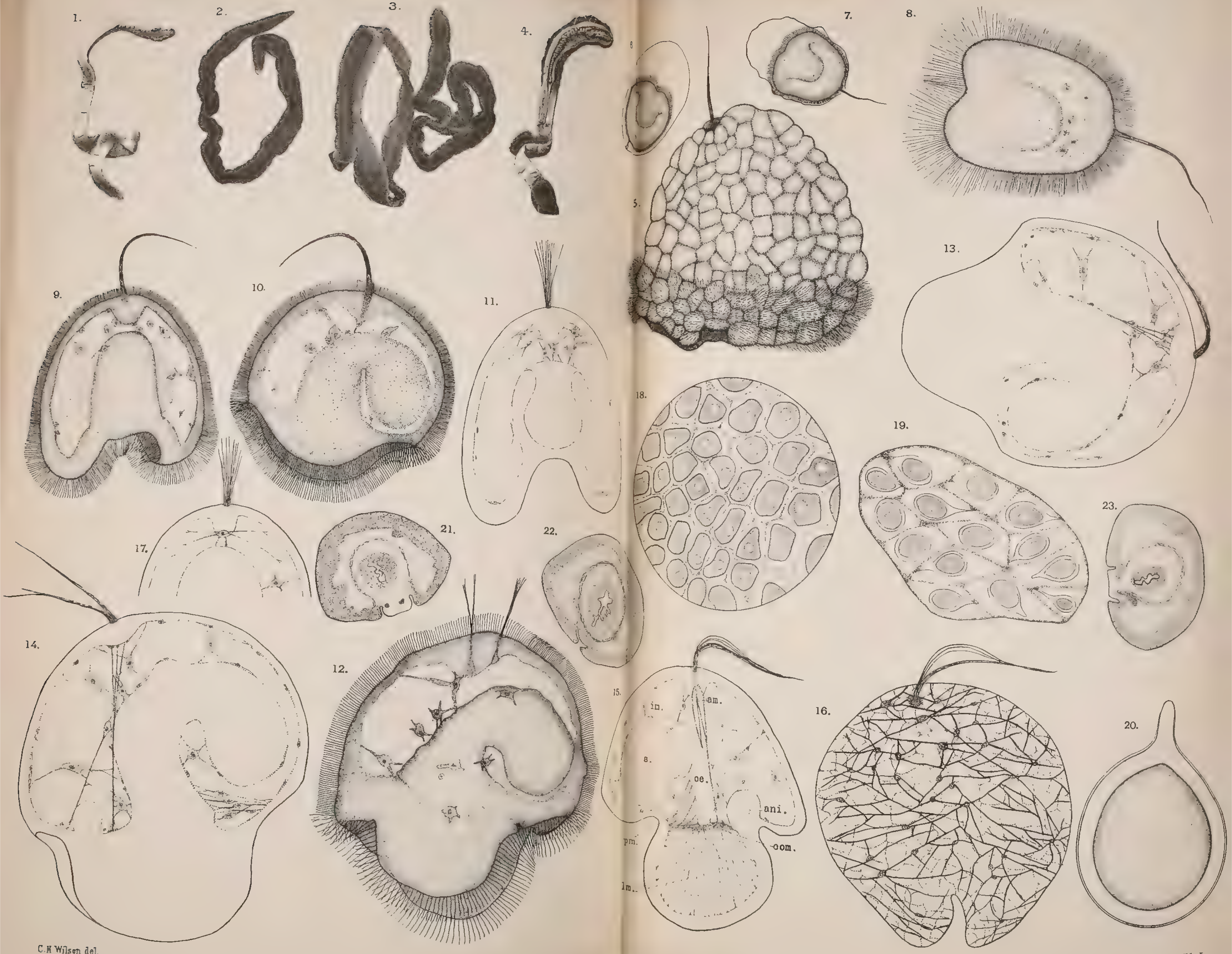


16.



20.

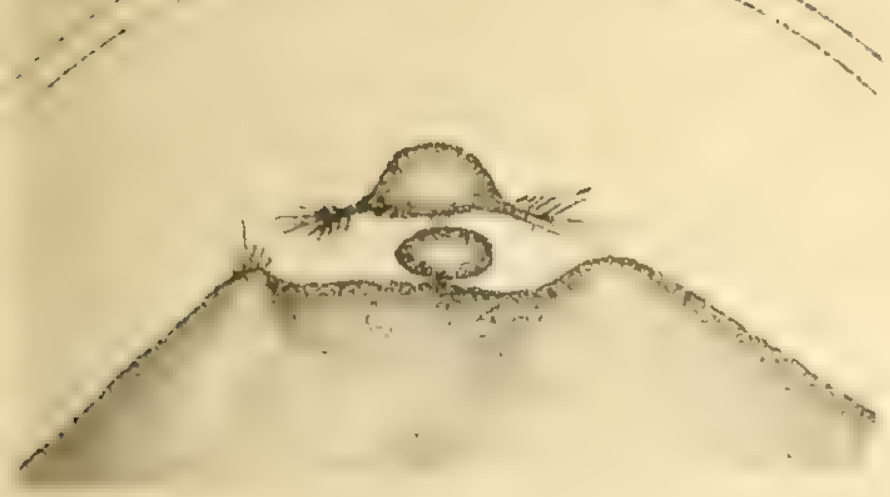




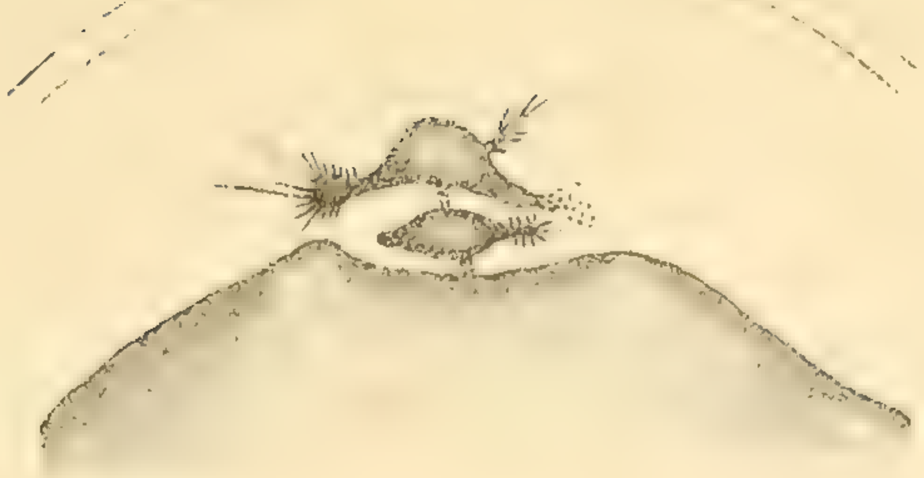
C. H Wilson del.

F. Huth, Lith^r Edin^r

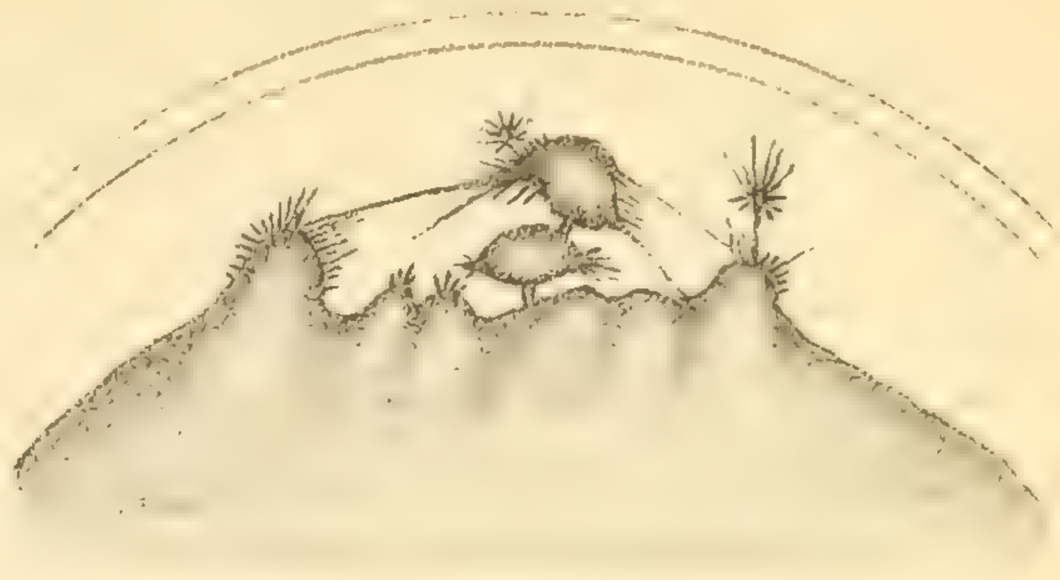
C.H.Wilson del.



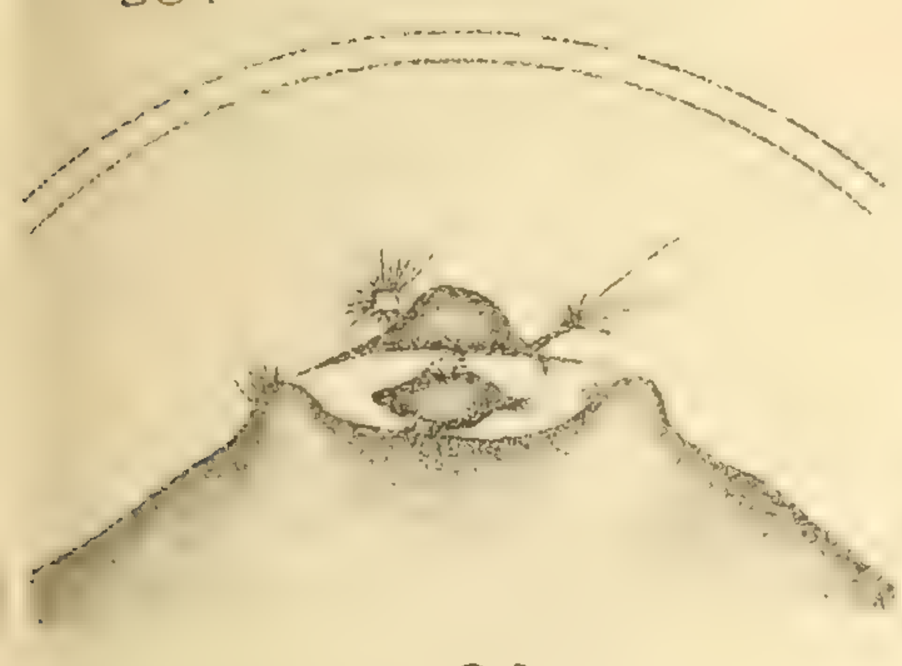
33.



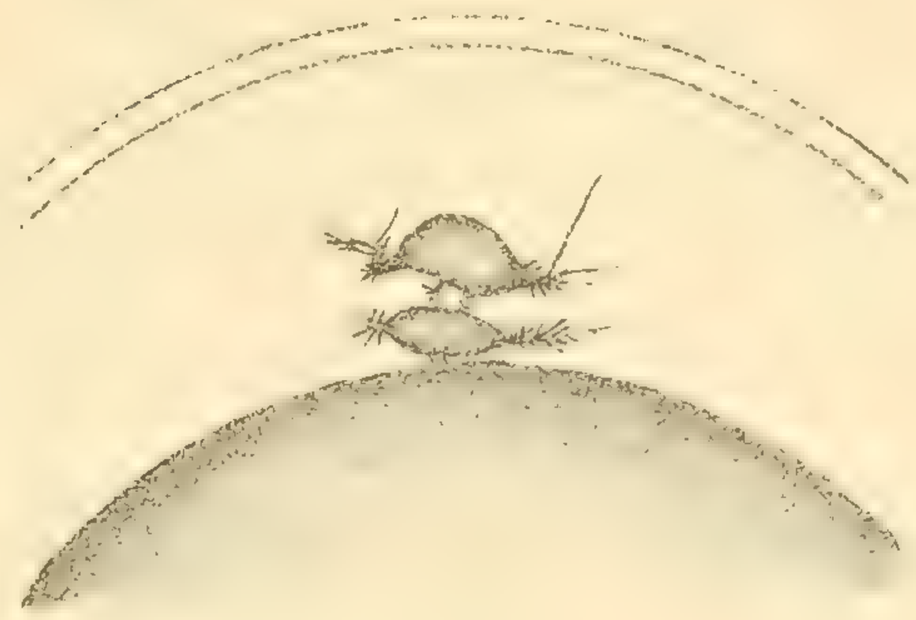
35.



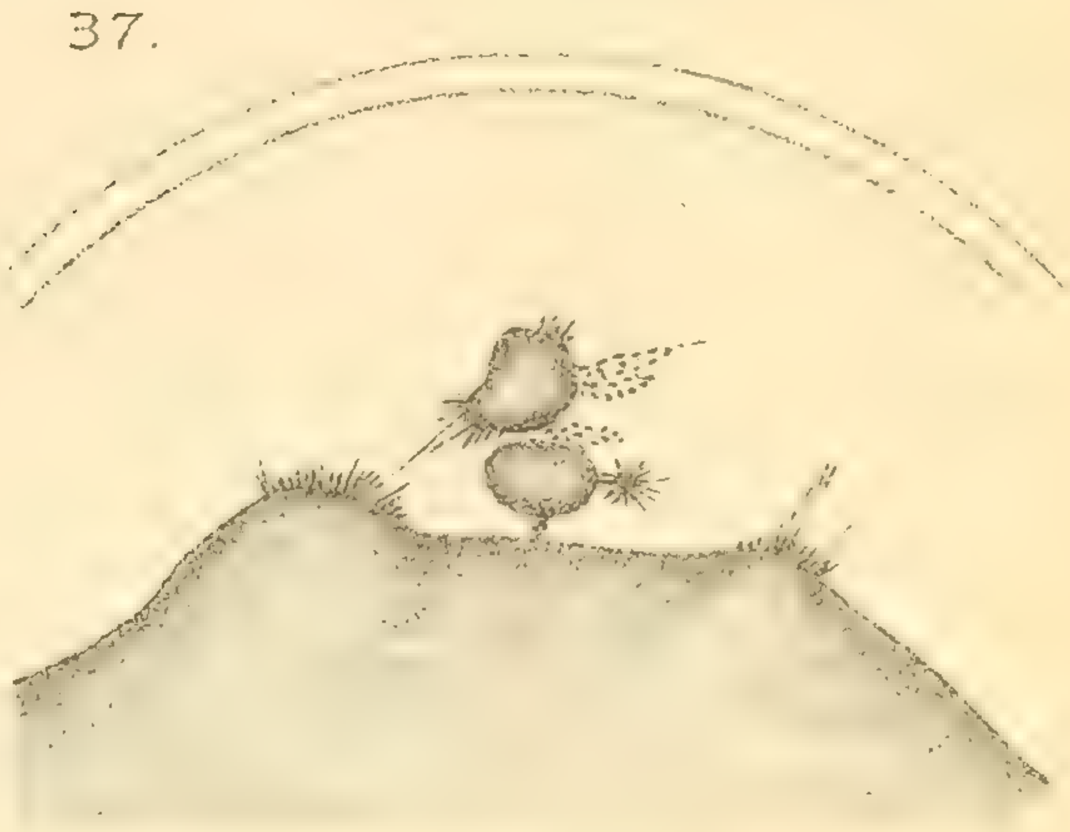
37.



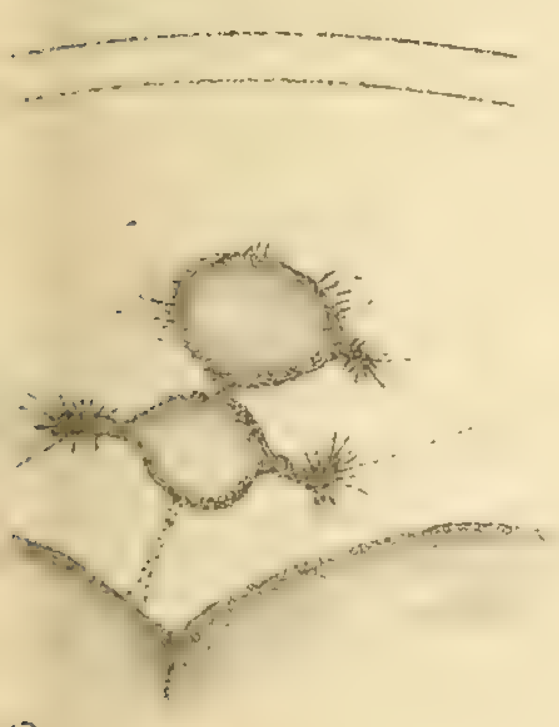
34.



36.



38.



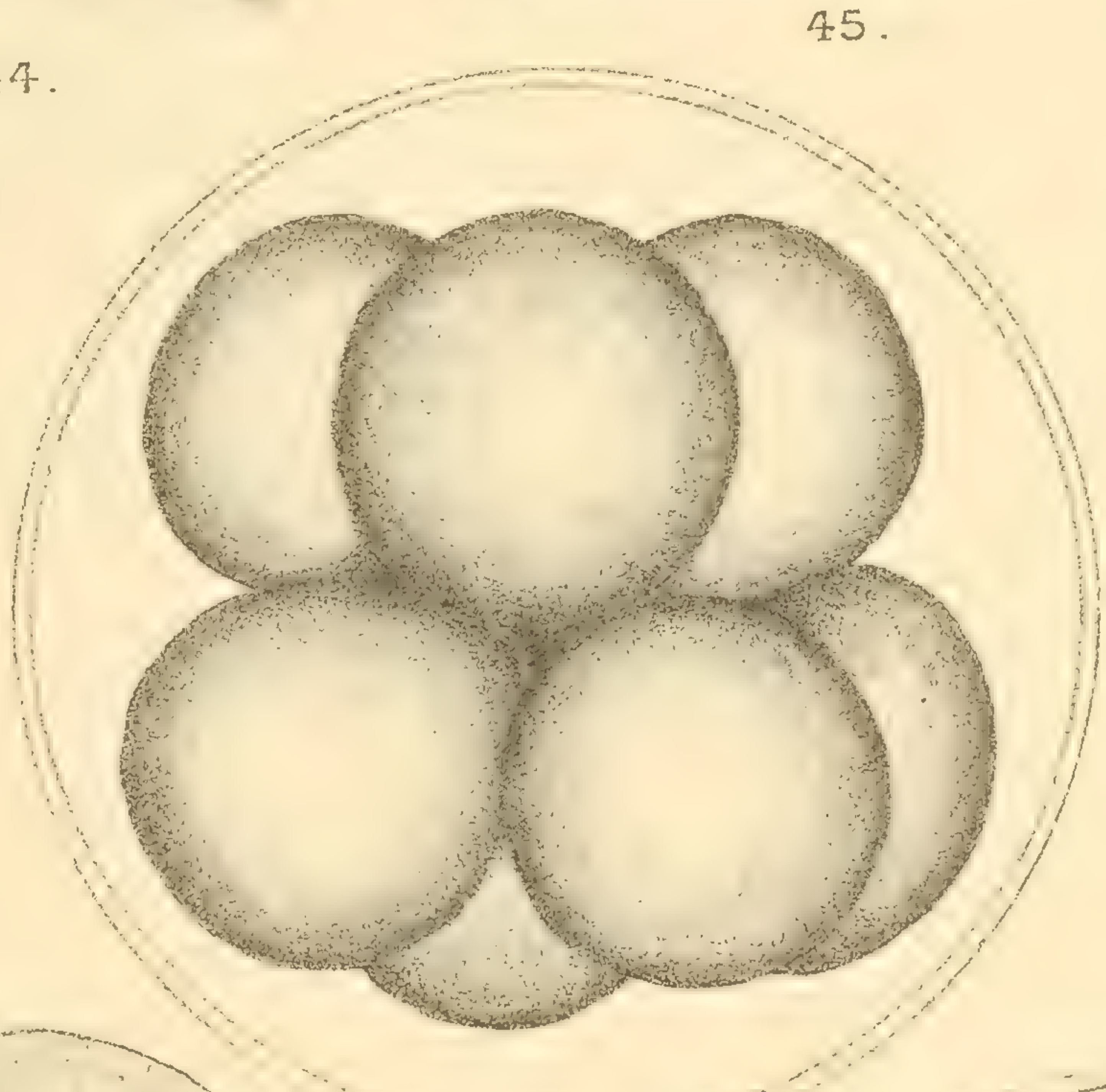
3.

44.

45.

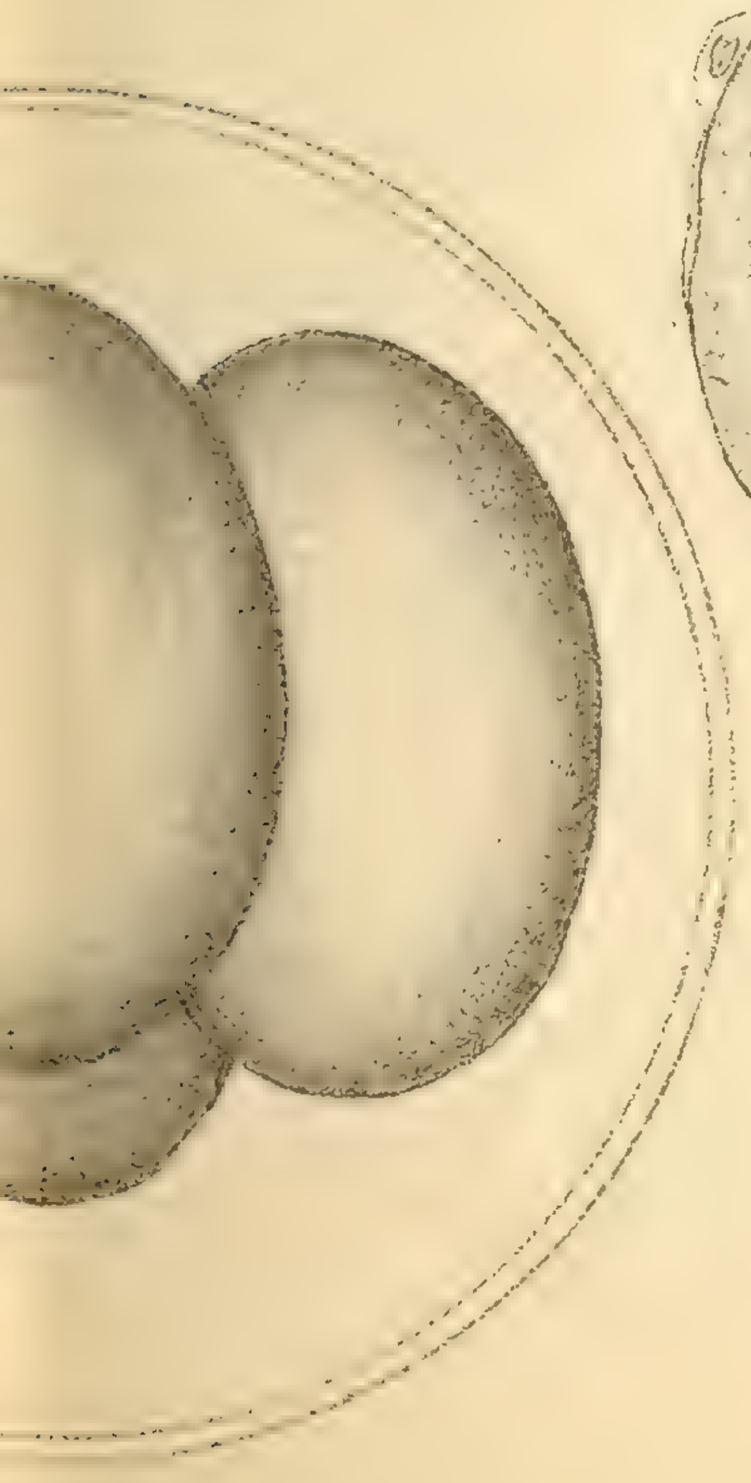
58.

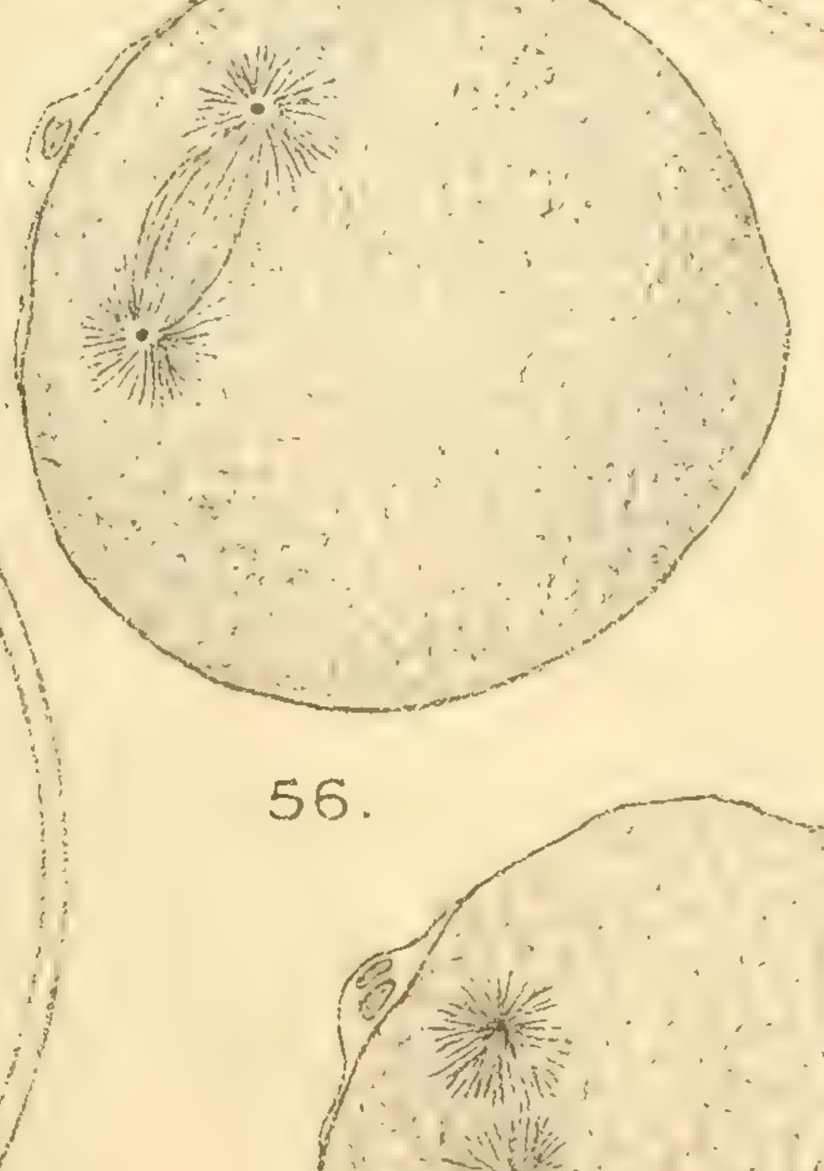
52.



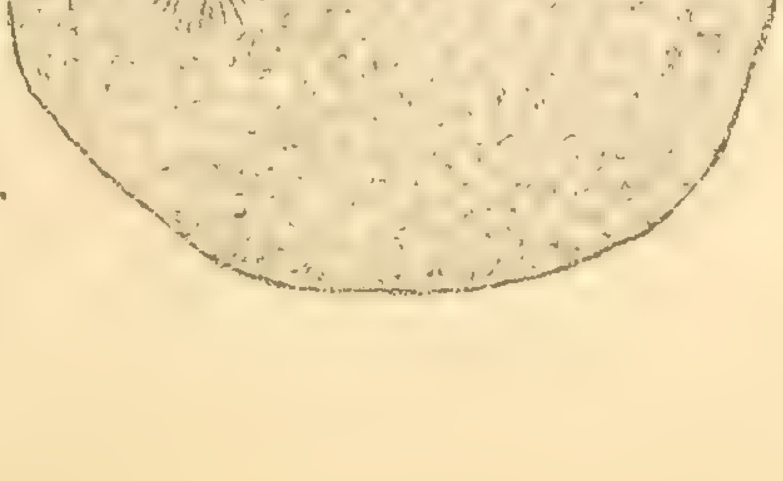
54.

59.

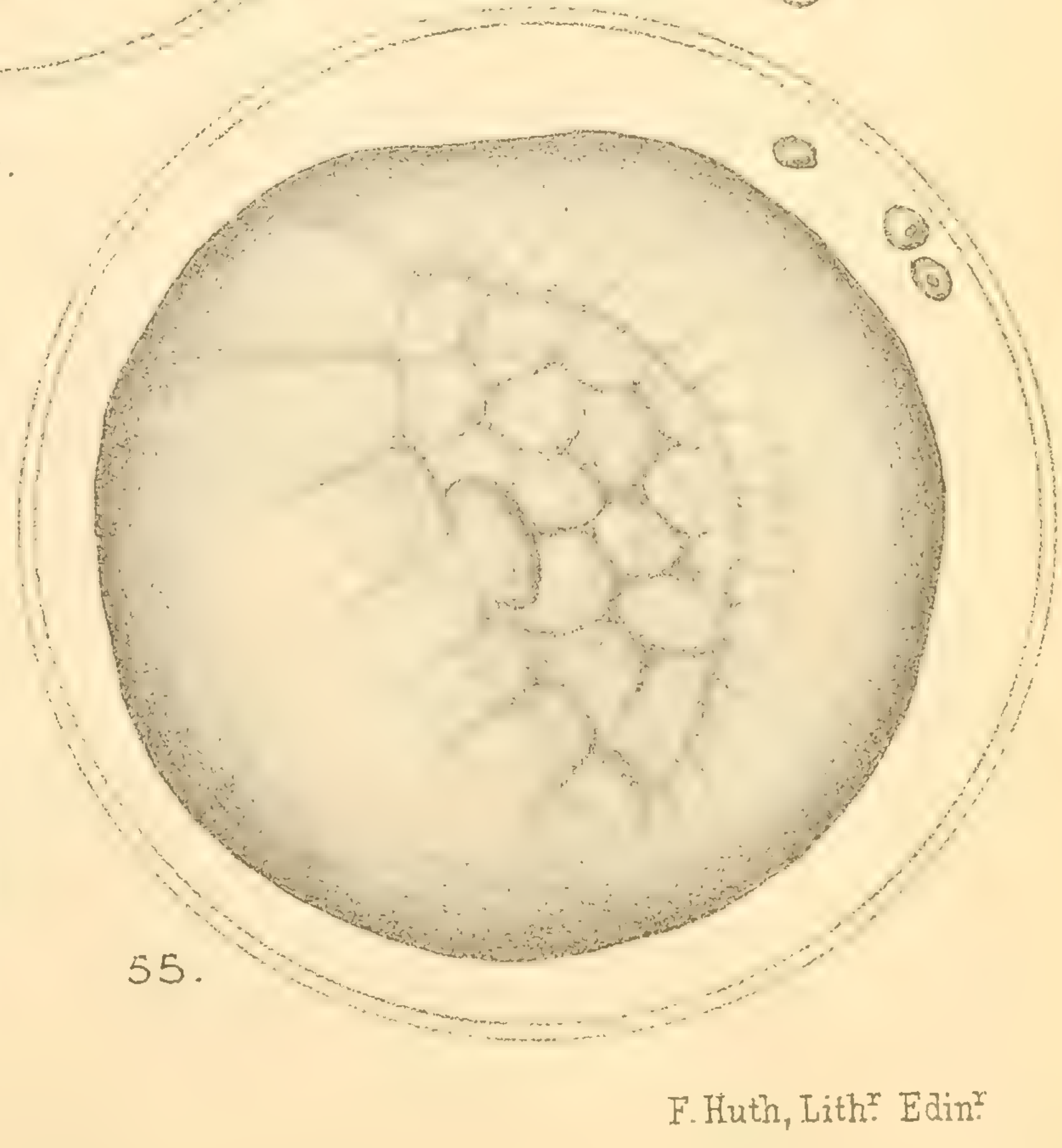




56.

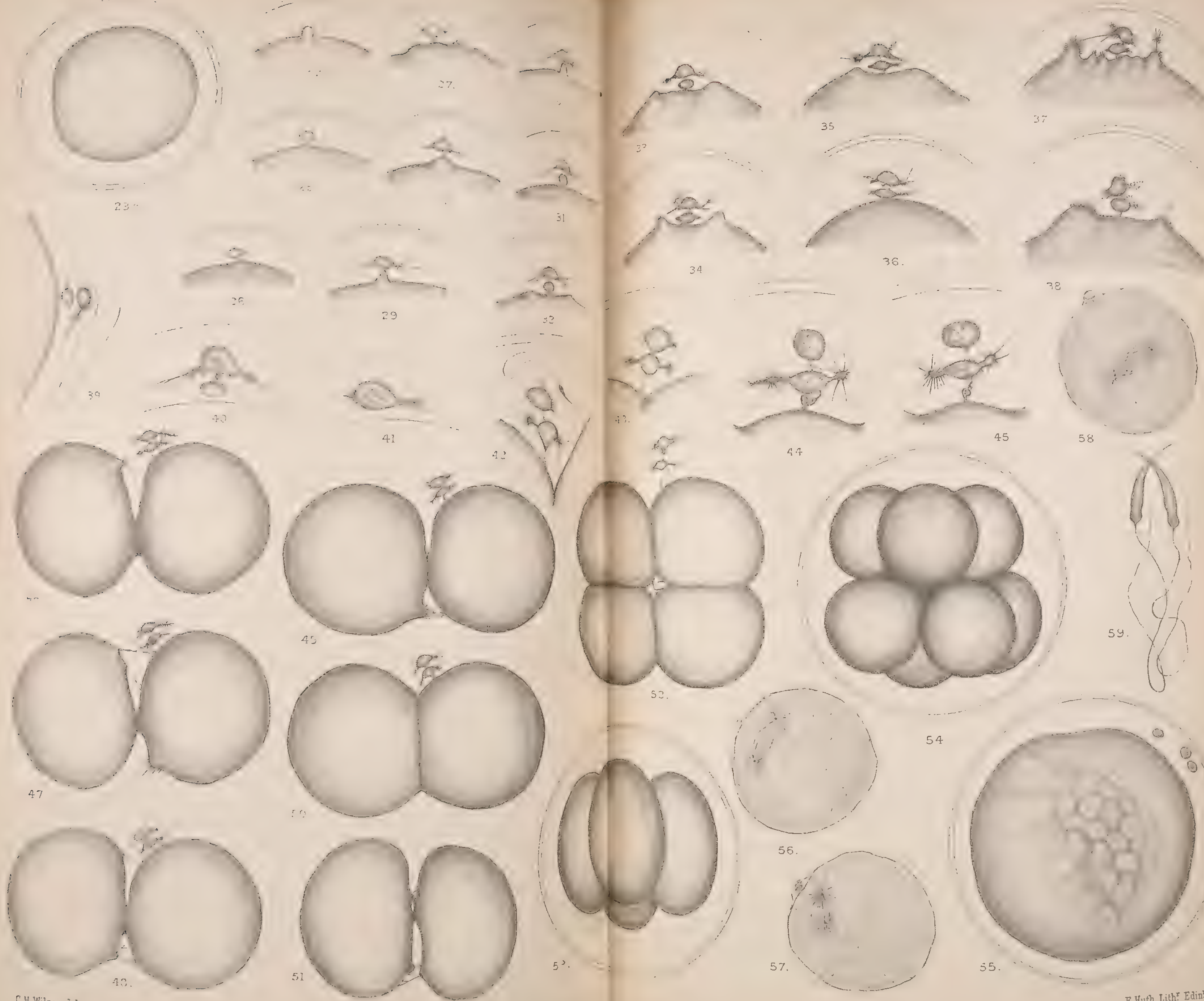


57.



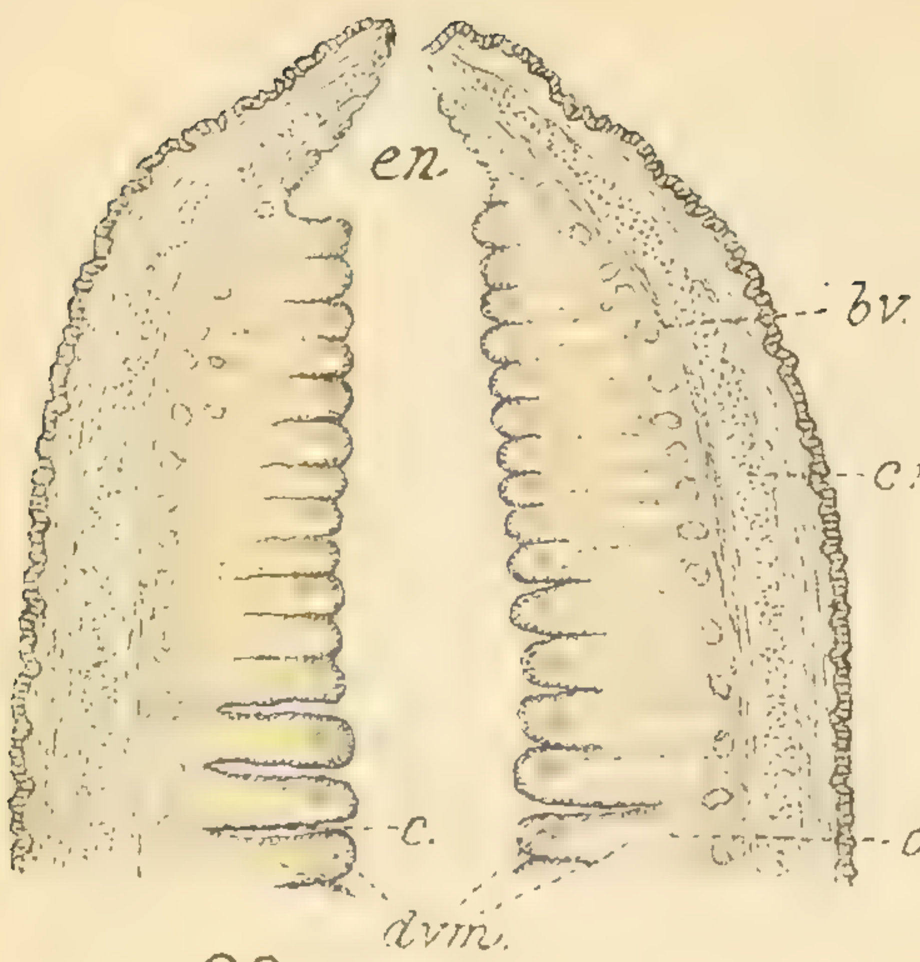
55.

F. Huth, Lith^r. Edin^r.

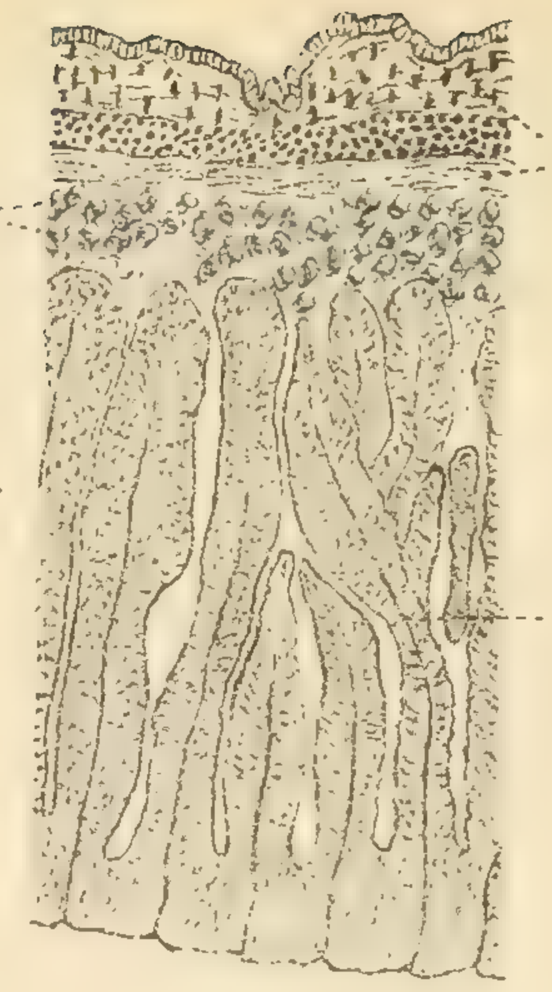


C H Wilson del

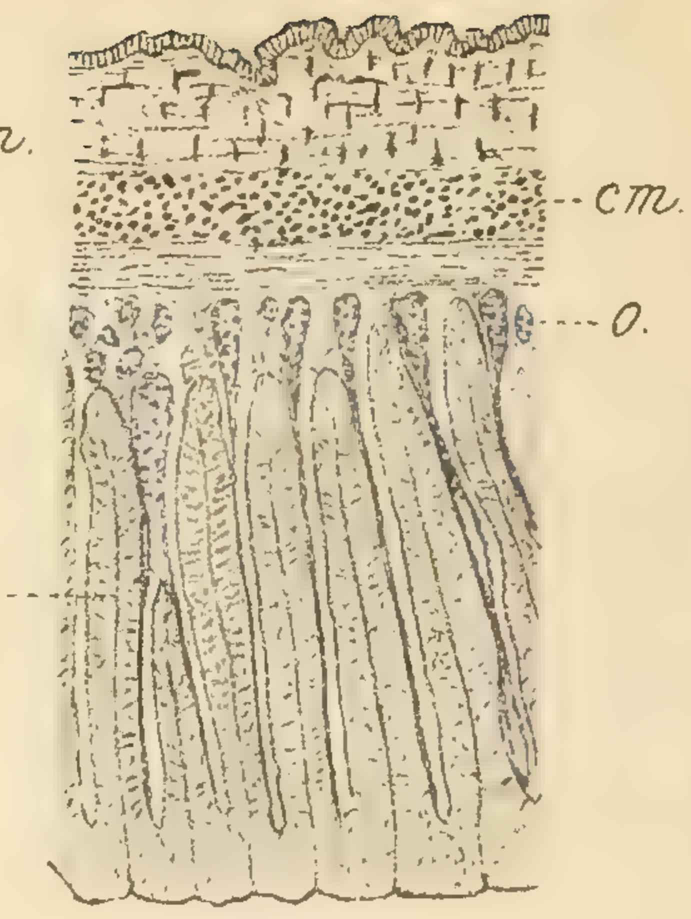
F Huth, Lith^{r} Edin^{r}



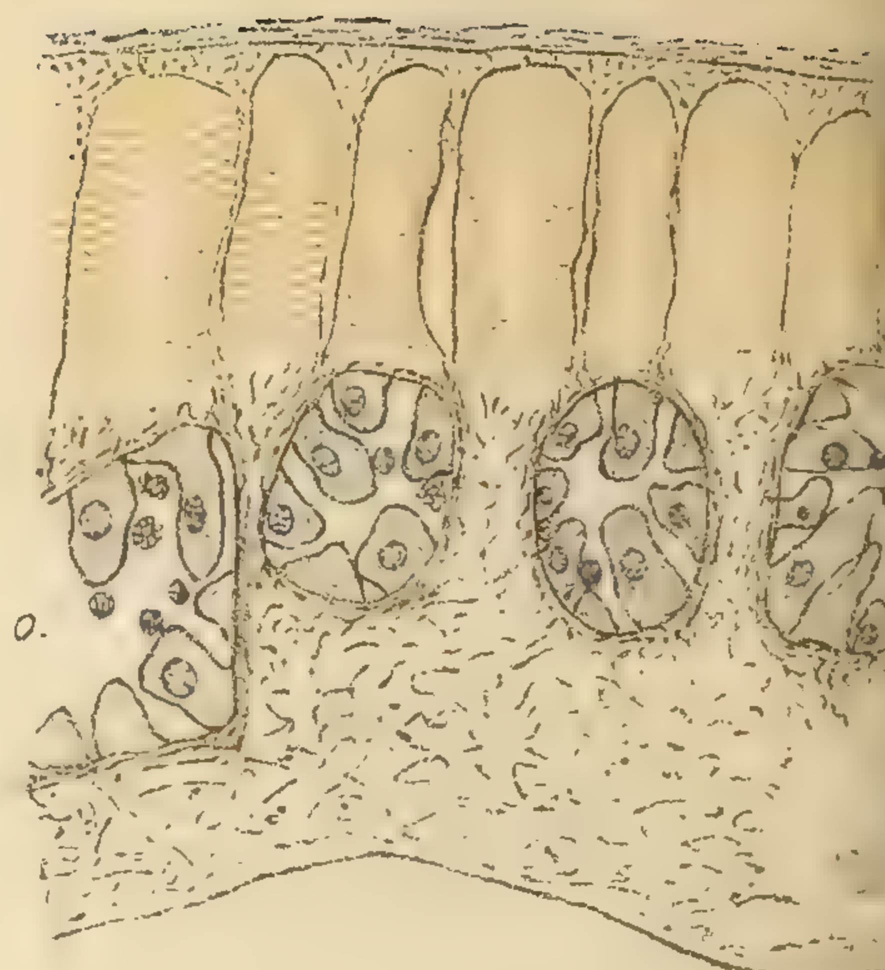
60.



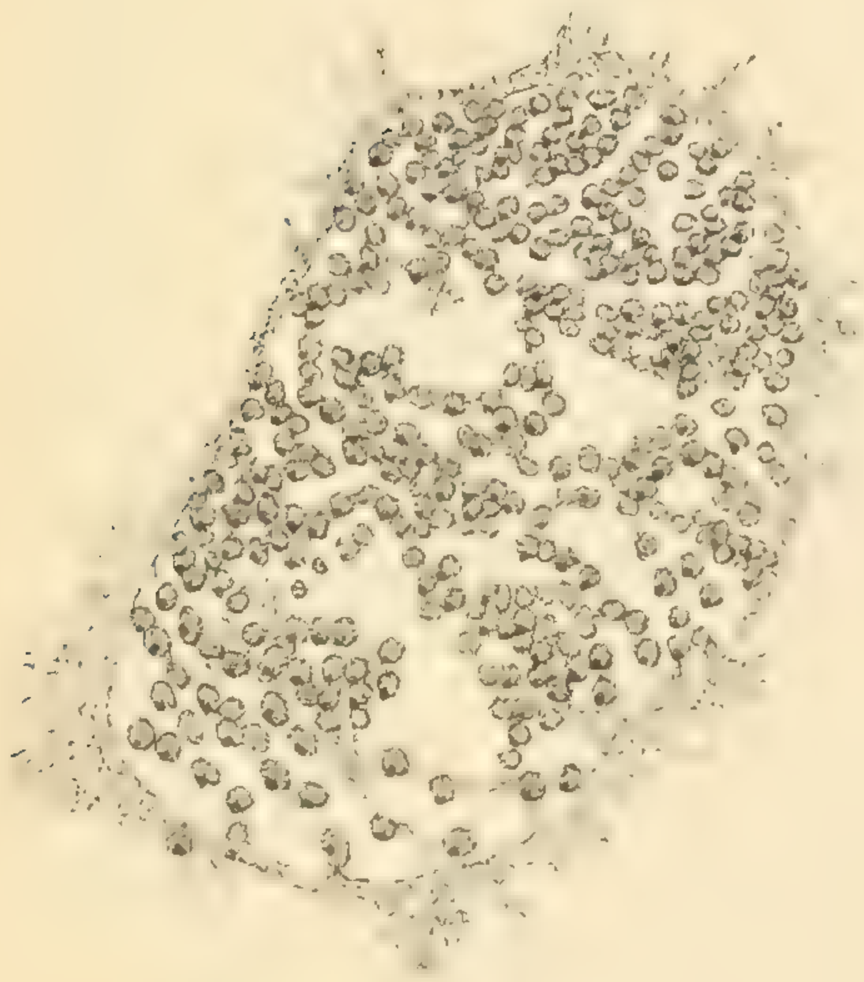
61.

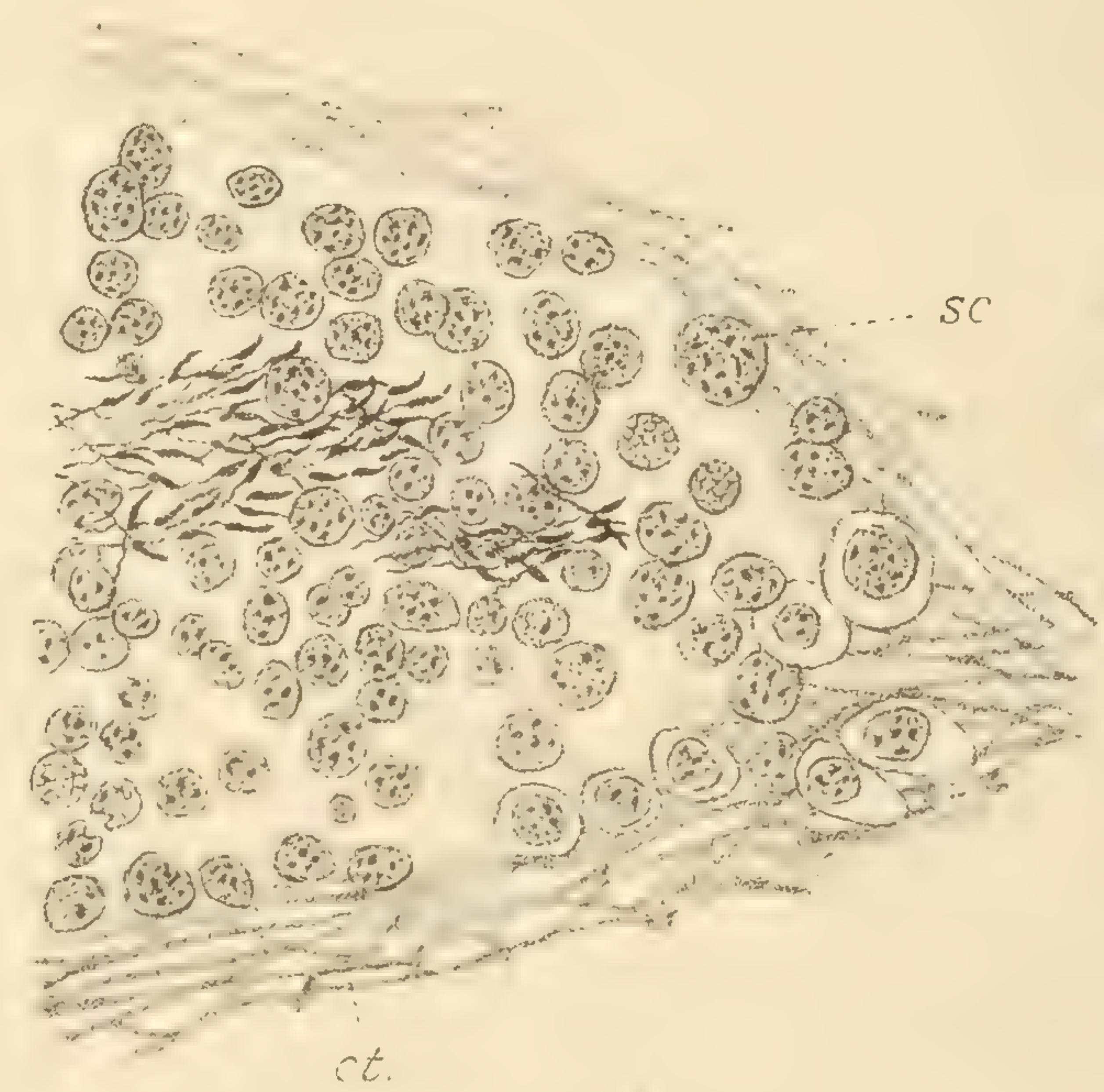


62.

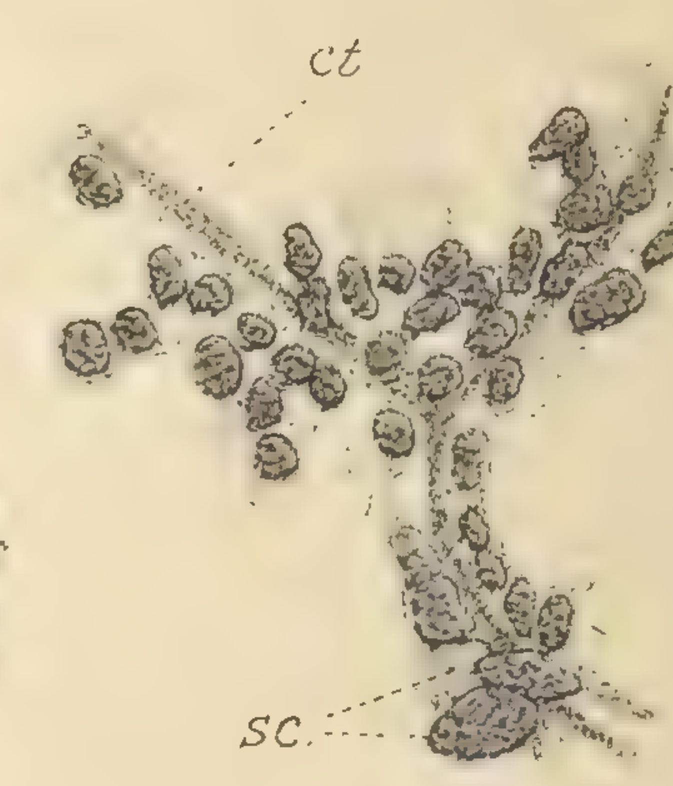


63.

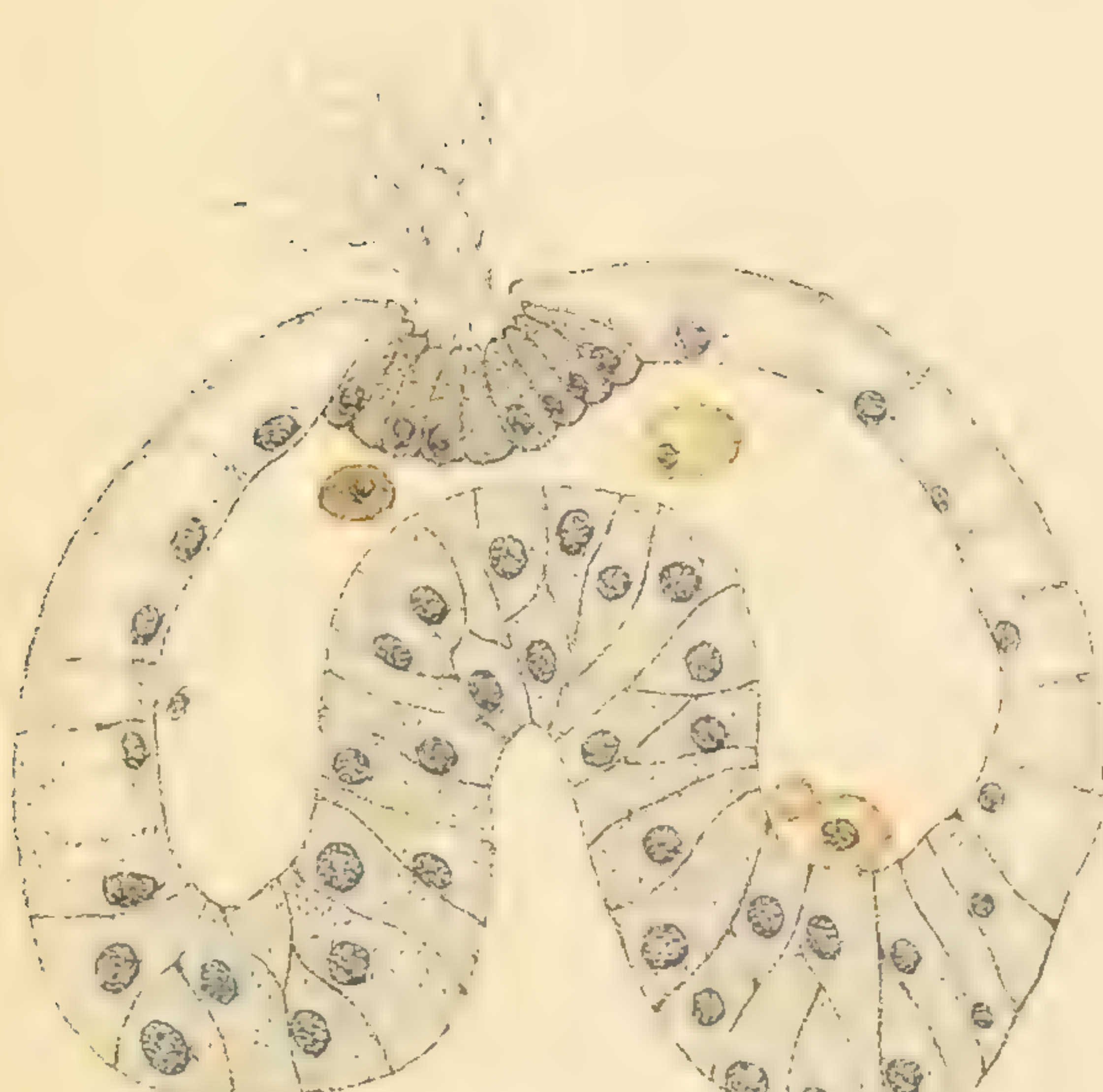

66.

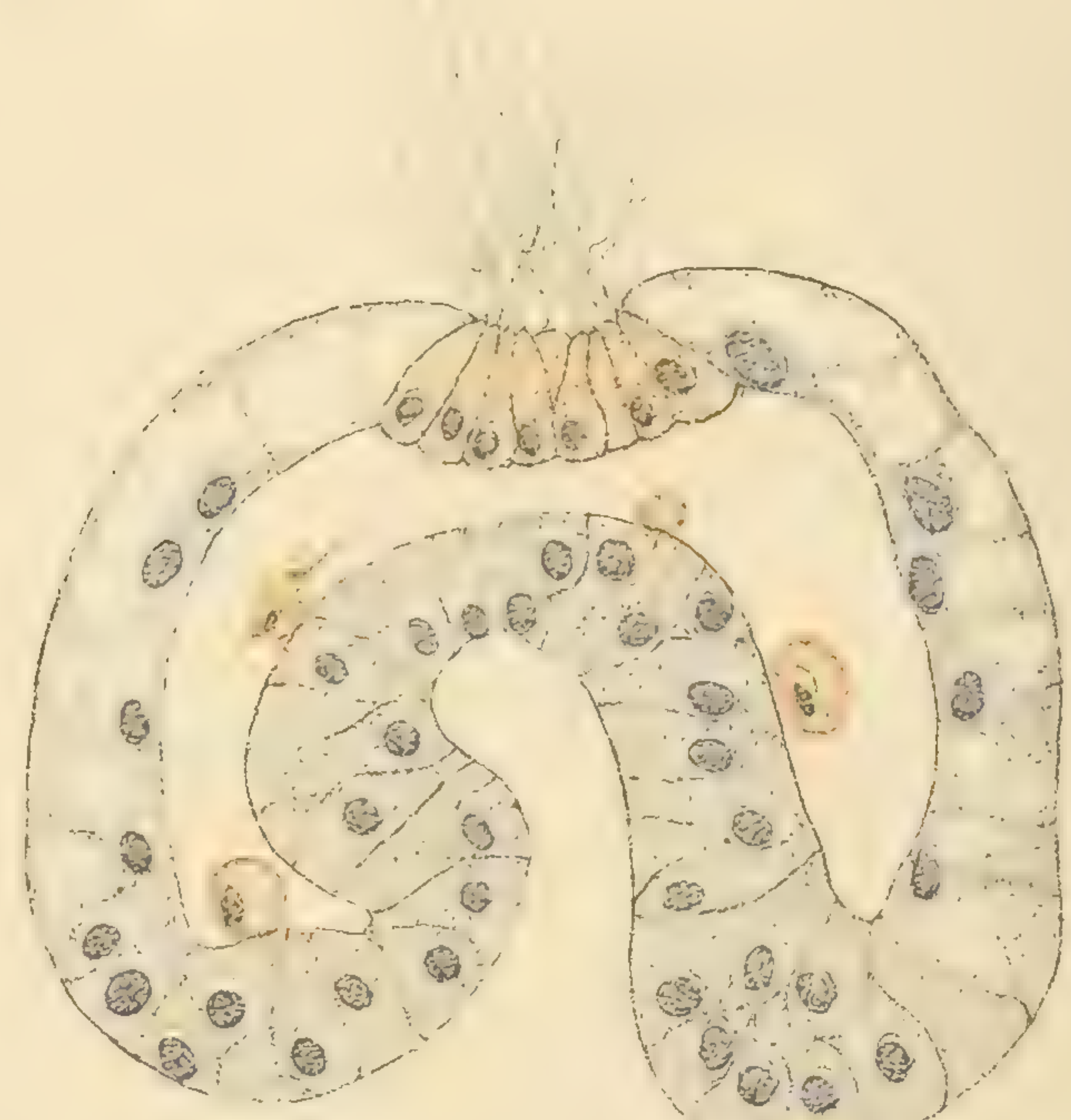


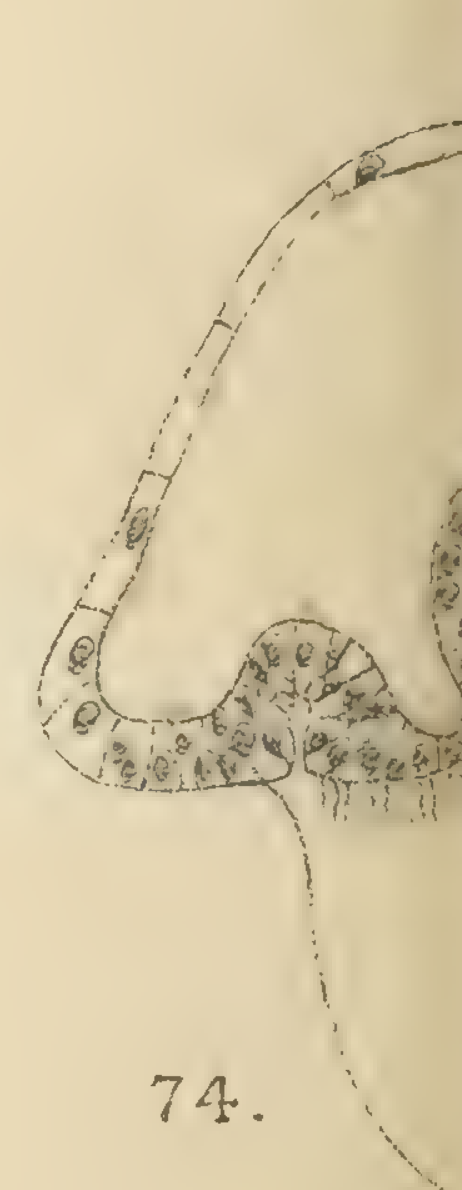
68.



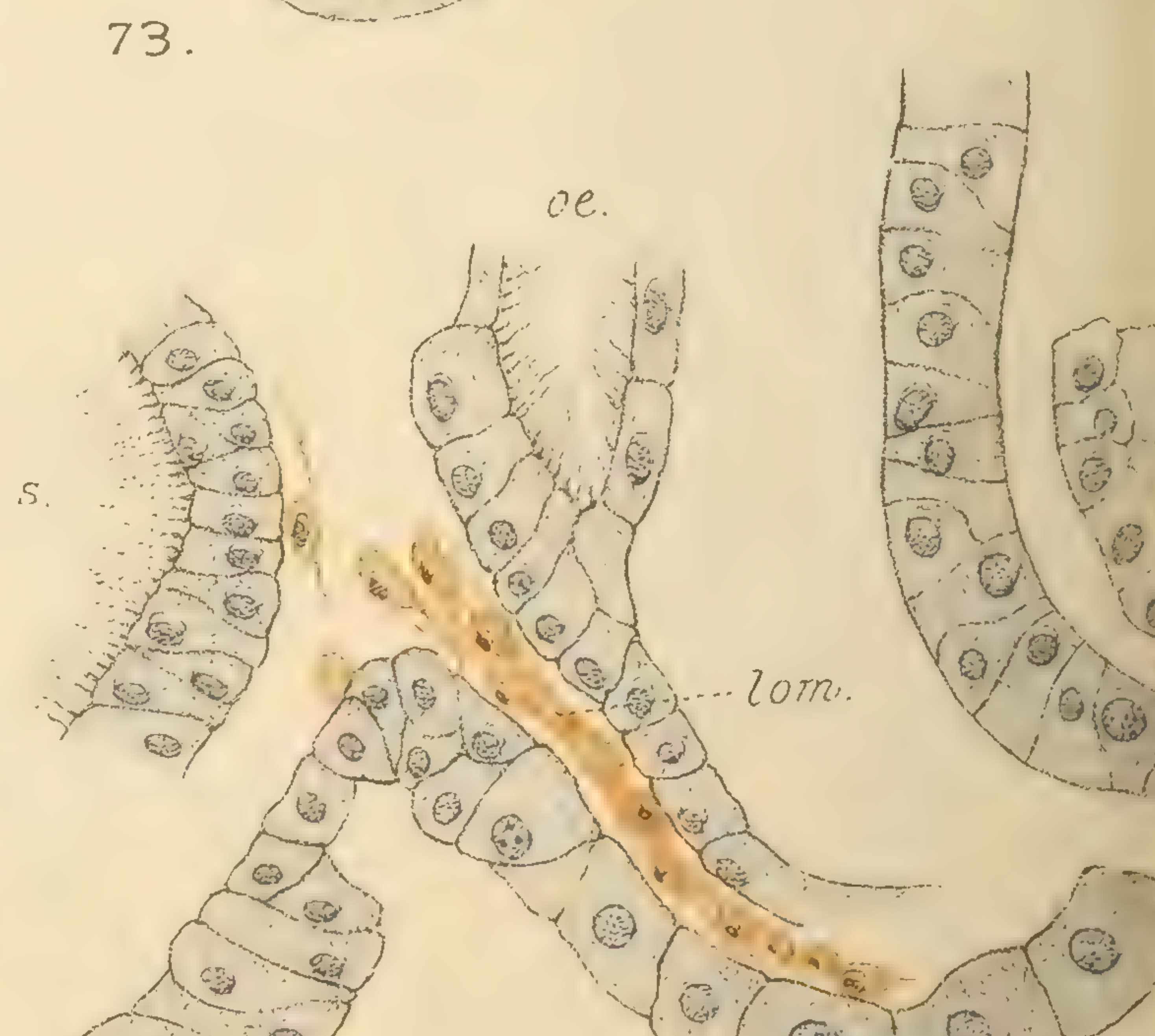
67.


72.


73.


74.

77.



79.

C.H. Wilson del.

F. Huth, Lith^r Edin^r

C. H. Wilson del.

F. Huth, Lith[r] Edin[r]